UNIVERSIDADE DE LISBOA

FACULDADE DE FARMÁCIA

ADVERSE EVENTS/MODE OF ACTION RELATIONSHIP OF MONOCLONAL ANTIBODIES-BASED THERAPIES: OVERVIEW OF MARKETED PRODUCTS IN THE EUROPEAN UNION

Francisca Mendes Lemos

Dissertation

Master's degree in Biopharmaceutical Sciences

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ABSTRACT

In the past few years, great discoveries and improvements have been done in drug development field and a market that was previously populated just by chemical drugs is now growing to include the so called biopharmaceuticals.

As biotechnologically-derived drugs rely on the fundamental understanding of the related disease, it can be predicted that they will play a major – if not dominant – role in the drug development arena of the next decades.

Following the success of recombinant proteins, therapeutic Monoclonal Antibodies (mAbs) represent the second wave of innovation created by the biotechnology industry. Overcoming the problems initially raised by these biopharmaceuticals, the recent generations of mAbs have managed to reduce some of the immunogenicity problems observed with murine mAbs. Other concerns remain, however, and the adverse events arising during the long-life experience will continue to be an important factor to monitor.

The target specificity of mAbs associated to the fact that they are large protein molecules make the emergence of off-target or metabolite–related toxicities less probable, being immunogenicity and target-mediated effects the most relevant determinants of toxicity.

This project focus was in the correlation and comparison of mAbs' adverse events with their specific mechanism of action. The data here analysed comprises mainly European data (EMA) but also some United States data [1]. Due to the large diversity of mechanisms for the marketed mAbs, the analysis has been restricted to three pharmacological classes of mAbs: anti-TNFα, anti-VGEF and anti-CD20 mAbs.

Adverse events were compared within each mAb class and then compared through all mAbs classes. There were antibody-related adverse events reported transversally for all mAbs but there were also some class-related adverse events which were only reported in specific classes, with specific mechanisms of action.

For class-related adverse events it was observed that additional key factors – like administration routes, mAbs structural configurations and profile of patients receiving those mAbs – may also impact the adverse events and cannot be excluded in safety profile characterisation.

It was confirmed that the adverse events reported for all mAbs analysed were strictly related to mAbs' mechanism of action. Hence, characterisation of each mAb specifities together with a precise understanding of the mAbs mechanism of action is crucial for mAbs safety profile characterisation.

Key Words: Biopharmaceuticals; Monoclonal Antibodies; mAbs Adverse Events; mAbs Mechanism of Action; anti-TNF alpha mAbs; anti-VEGF mAbs; anti-CD20 mAbs.

LIST OF ACRONYMS

Abs	Antibodies
ADCC	Antibody-Dependent Cell-Mediated Cytotoxicity
ADCP	Antibody-Dependent Cellular Phagocytosis
AE	Adverse Event
AMD	Age-Related Macular Degeneration
ANA	Antinuclear Antibodies
APCs	Antigen-Presenting Cells
AS	Ankylosing Spondylitis
ASAS	Assessment In Ankylosing Spondylitis
ATC	Anatomical Therapeutic Chemical Classification System
AxSpA	Axial Spondyloarthritis
BLA	Biologic License Application
BRB	Blood Retinal Barrier
BRVO	Branch Retinal Vein Occlusion
CBER	Center For Biologics Evaluation And Research
CD	Crohn's Disease
CD20	B-Lymphocyte Antigen
CDC	Complement-Dependent Cytotoxicity
CDER	Center For Drug Evaluation And Research
CDR	Complementarity-Determining Region
CLL	Chronic Lymphocytic Leukaemia
CNV	Choroidal Neovascularisation
CRP levels	C-Reactive Protein
CRVO	Central Retinal Vein Occlusion
DC	Dendritic Cells
DME	Diabetic Macular Oedema
DNA	Deoxyribonucleic Acid
ds-DNA	Double-Stranded DNA
EMA	European Medicines Agency
EOC	Epithelial Ovarian Cancer
EPAR	European Public Assessment Report
EU	European Union
Fab	Fragment Antigen Binding Region
FAERS	FDA Adverse Events Reporting System
Fc	Fragment Crystallisable
FDA	Food And Drug Administration
FTC	Fallopian Tube Cancer
GPA	Granulomatosis With Polyangiitis
HAMA	Human Anti-Mouse Antibodies
HLGT	High-Level Group Terms (MedDRA classification)
HPV	Human Papillomaviruses
IgG	Immunoglobulin

LPS	Lipopolysaccharide
MA	Marketing Authorisation
mAb	Monoclonal Antibody
mBC	Metastatic Breast Cancer
MCP	Metacarpophalangeal
mCRC	Metastatic Carcinoma Of The Colon Or Rectum
MedDRA	Medical Dictionary For Regulatory Activities
MeSH	Medical Subject Headings
MHC	Major Histocompatibility Complex
MPA	Microscopic Polyangiitis
mRCC	Metastatic Renal Cell Carcinoma
NDA	New Drug Application
NHL	Non-Hodgkin's Lymphoma
NK	Natural Killer Cells
NSCLC	Non-Small Cell Lung Cancer
PAMPs	Pathogen-Associated Molecular Patterns
PIP	Proximal Interphalangeal
Poly-JIA	Polyarticular Juvenile Idiopathic Arthritis
PPC	Primary Peritoneal Cancer
Ps	Psoriasis
PsA	Psoriatic Arthritis
R&D	Research And Development
RA	Rheumatoid Arthritis
rDNA	Recombinant DNA
RF	Rheumatoid Factor
RNA	Ribonucleic Acid
RVO	Retinal Vein Occlusion
SmPC	Summary Of Product Characteristics
SOC	System Organ Class (MedDRA classification)
TNF	Tumour Necrosis Factor
TNFα	Tumour Necrosis Factor Alpha
U.S.	United States
UC	Ulcerative Colitis
VA	Visual Acuity
VEGF	Vascular Endothelial Growth Factor

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INTRODUCTION

From a pharmacological point of view, the ideal drug would be the one with a specific pathology-related target, a precise and effective mechanism of action, no systemic side effects and efficient elimination. Although very wanted, no drug currently available has such high specificity and low toxicity. In the beginning of the 20th century, the German immunologist and Nobel Prize winner Paul Ehrlich figured that «*if a compound could be made to selectively target a disease causing organism, then a toxin for that organism could be delivered along with the agent of selectivity*» and introduced, this way, the idea of "magic bullet" against disease. Nevertheless, once again, he also said that magic substances like antibodies which affect exclusively the harmful agent would not be easy to find [2, 3]. Due to their ability to selectively hit a specific target, monoclonal antibodies (mAbs) may be the closest fit to the concept of "ideal drugs" or "magic bullets", first proposed by Paul Ehrilich [2-5].

I. Biopharmaceuticals

1. Historic Note – From small molecules to biopharmaceuticals

In the past few years, great discoveries and improvements have been done in drug development filed and a market that was previously populated just by chemical drugs is now growing to include the so called biopharmaceuticals [6-9]. Also referred in literature as "biologicals", "biological medicinal products", "biotechnologically-derived drugs" or just "biologics", these drugs are revolutionizing treatment for many diseases [5, 7, 10, 11].

According to FDA, therapeutic biologic products or biologics can be defined as *"a virus, therapeutic serum, toxin, antitoxin, vaccine, antibody, blood, blood component or derivative, allergenic product, or analogous product, or arsphenamine or derivative of arsphenamine (or any other trivalent organic arsenic compound), applicable to the prevention, treatment, or cure of a disease or condition of human beings"* [1]. Biopharmaceuticals are a subset of drug products distinguished by their manufacturing process. While classic drugs (chemicals) are synthesized via a chemical process, biopharmaceuticals are manufactured utilizing biological process (e.g. living cells) and are typically derived from living material – as human, animal, or microorganism. Biotech drugs can be considered as those biopharmaceuticals that are manufactured using biotechnology-based production process [5]. Apart from obvious differences in manufacturing process, some other great differences can be found between these two sets of therapeutic molecules (biopharmaceuticals *vs.* traditional chemicals) and those include, but are not limited to, size, complexity and efficacy, which cannot be compared to chemical's, mainly due to high specificity of biopharmaceuticals [7-9, 12-16].

In recent years, biotechnologically-derived drugs including proteins, peptides, monoclonal antibodies and antibody fragments, have been a major focus of research and development (R&D) efforts in the pharmaceutical industry and these biopharmaceuticals constitute already a sizable fraction of the medications used in clinical practice [5]. It has been estimated that more than 250 million patients have benefited from a biopharmaceuticals to treat or prevent heart attacks, stoke, multiple sclerosis, leukaemia, hepatitis, rheumatoid arthritis, breast

cancer, diabetes, congestive heart failure, kidney cancer, cystic fibrosis and other diseases [5]. Since the development of biotechnologically-derived drugs generally rests on a fundamental understanding of the related disease, clinical development of biopharmaceuticals has potential to be more successful than conventional chemically derived drugs. It is predictable that biotechnologically-derived drugs will play a major – if not dominant – role in the drug development arena of the next decades [5, 17]. Interferons to treat multiple sclerosis, anti tumour necrosis factor to treat cancer or epoetin alpha to stimulate red blood cell production, are examples of biopharmaceuticals [16].

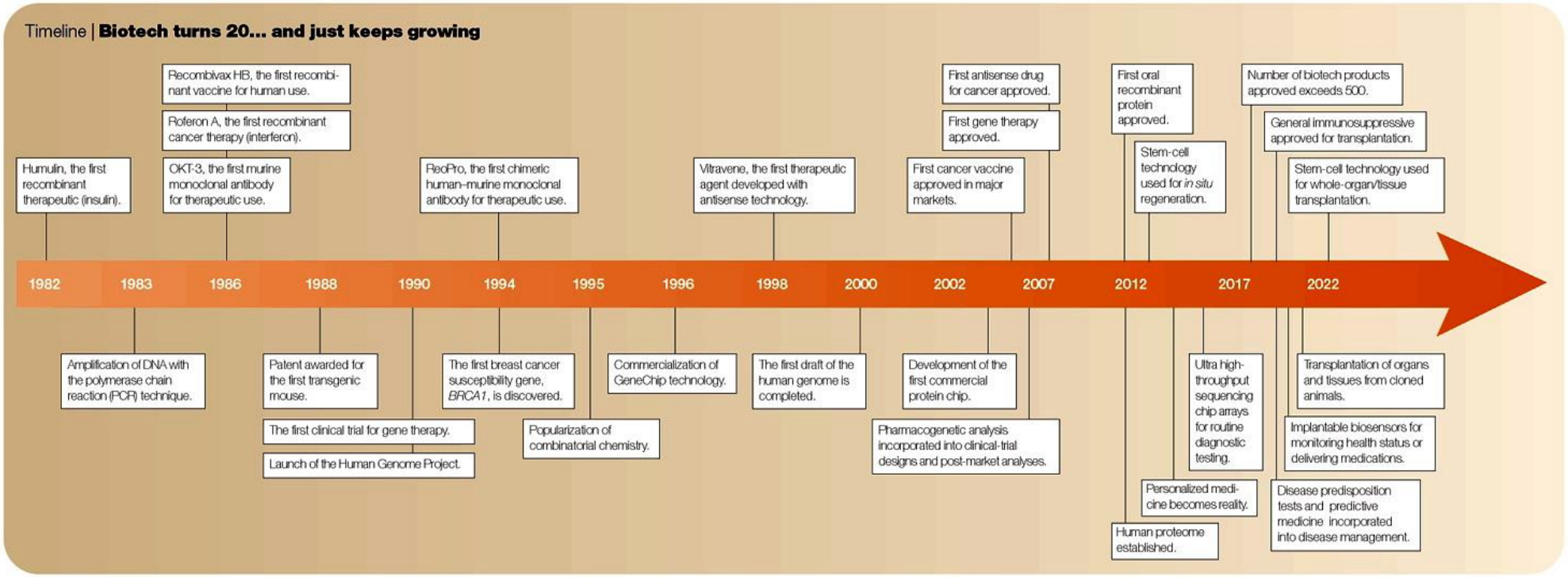

Figure 1 – Advances in biotech drug development. ***In Tricia Nagle et al., 2003*****.**

2. Biotechnology – at first, all were replacing therapies

Biotechnology, founded on the principles of recombinant DNA (rDNA) protein production is an R&D-intensive sector. Beginning in the early 1970s, advances in molecular biology and genetic engineering have led to enormous progress in the ability to understand the biomolecular roots of human disease.

Stanford University's Paul Berg, the 1980 Nobel Prize winner in chemistry, first produced rDNA in 1972. His lead was followed by a team coordinated by Herbert Boyer (University of California, San Francisco) who, in 1973, transformed Escherichia coli cells with recombinant plasmid and later founded Genetic Engineering Technology (Genentech, S. San Francisco, CA, U.S.).

In 1978, the Genentech joined City of Hope National Medical Center to produce human insulin in the laboratory using recombinant DNA (rDNA) technology. City of Hope National Medical Center' scientists had synthesized the genes for the protein's two chains before inserting them into *Escherichia coli*, which would, in turn, be stimulated to synthesize insulin.

With its expertise in purifying and handling insulin and the desire to remain a leader in the field, Eli Lilly (Indianapolis, IN, U.S.), immediately licensed recombinant insulin from Genentech and set about developing it. Because rDNA guidelines at the time allowed the expression of only inactive protein products, Eli Lilly had to use the two-chain method. Clinical studies began in 1980 and in 1982, Eli Lilly's recombinant human insulin (Humulin), became the first genetically engineered drug approved in the United States and the United Kingdom [18-20].

FDA Endocrine Division Director Dr. Sol Sobel signed the FDA's HUMULIN approval letter. He was not unaware of the historical significance of this letter, *«Except for my marriage license»* he recalls, *«that* [approval letter] *was the most important document I ever signed»* [19].

The US approval came just one month after British regulatory authorities allowed its introduction in the UK. Although the development of HUMULIN took just four years, it was a major undertaking for both the developers and regulators, breaking new ground. The reason for the speedy action in the United States, despite the newness of the product, was attributed to *"the vast amount of prior experience with the animal insulins, which are very closely related to HUMULIN"*, said Dr. Miller (medical officer in charge of HUMULIN at the FDA) [21]. Animal and laboratory studies have shown that the potency of the genetically engineered human insulin appeared to be virtually indistinguishable from that of the purified pork insulin.

Despite initial reservations about the technology, rDNA derived proteins actually had an enhanced safety profile and HUMULIN had proved to be safe and effective in clinical trials involving more than 400 patients [18, 19].

After more than two decades of continuous global expansion, business formation, technological diversification as well as subsequent approvals of crucial rDNA products – such as the rDNA form of an enzyme to treat Gaucher's disease, thyroid stimulating hormone (TSH) for diagnostic use in thyroid cancer, as well as many products for the production of monoclonal antibodies for the treatment of cancer – the sector of rDNA therapeutics is now the core of the human medical biotechnology industry [18, 19, 22]. *«At the present time»*

concludes FDA's Dr. Sol Sobel, *«I can't imagine producing a new therapeutic protein by any other means»* [19].

3. Regulatory Approval Process

EUROPE: In the European Union (EU), medicines can be authorised by the centralised authorisation procedure or national authorisation procedures. Biopharmaceuticals are all approved by centralised authorisations [23].

As per Regulation (EC) No 726/2004, centralised procedure in Europe is compulsory for medicinal products developed by means of biotechnological processes like: recombinant DNA technology; controlled expression of genes coding for biologically active proteins in prokaryotes and eukaryotes including transformed mammalian cells; and hybridoma and monoclonal antibody methods [24].

Applications through the centralised procedure are submitted directly to the Agency and once a marketing authorisation has been granted, the marketing-authorisation holder can legally begin to market the medicine in all EU countries, as well as in the European Economic Area (EEA) countries Iceland, Liechtenstein and Norway [23].

UNITED STATES: Similarities in the drug development and evaluation process for biotech drugs and conventional, chemical synthesized drugs have recently been acknowledged in the FDA's 2003 decision to transfer certain product oversight responsibilities from the **C**enter for **B**iologics **E**valuation and **R**esearch (CBER) to the **C**enter for **D**rug **E**valuation and **R**esearch (CDER). The biologics whose oversight was transferred include monoclonal antibodies for in vivo use, proteins intended for therapeutic use, including cytokines (e.g. Interferons), enzymes (e.g. thrombolytic), growth factors, and other novel proteins that are derived from plants, animals, or microorganisms, as well as recombinant versions of these products, and other non-vaccine and non-allergenic therapeutic immunotherapy. Classical biologics such as blood, blood components and vaccines remain under the regulatory authority of the CBER.

Although under this new structure, the biologic products transferred to CDER will continue to be regulated as licensed biologics – meaning a Biologic License Application (BLA) must be submitted to obtain market-authorization, in opposition to a New Drug Application (NDA) which is used for traditionally, chemically manufactured drug products [5].

4. Biosimilars

With the biopharmaceutical's market quote growing, together with the fact that a substantial number of biopharmaceutical's patents are starting to expire, the so-called copycat pharmaceuticals aroused interest in developing generics of the biopharmaceuticals [6-8, 25]. These new medicines seek to copy the effect and activity of innovator biological medicines and with the advantage of less associated costs [6, 7]. However, the aim to copy the effect, cannot be pursued using a conventional generic manufacture process [12, 25, 26].

As proteins, biologicals present many changes in activity and compound interactions within the body when compared to traditional medicines [25]. For this reason, and bearing in mind that some of those interactions are not even well explained yet, the assessment and approval cannot be strictly based on pharmacokinetic assessments of bioequivalence. Even the tiniest change in the manufacture process can lead to changes in the structure of the medicine which, ultimately, may lead to differences in its biological activity, elimination, distribution and metabolism, leading to consequent alterations of efficacy, toxicology and ability to trigger patient immune response [7, 12-14, 16].

Biosimilars are a new class of biological medicines [15] and, according to EMA, defined as:

BIOSIMILAR: "biological medicinal product that contains a version of the active substance of an already authorised original biological medicinal product (reference medicinal product) in the EEA. Similarity to the reference medicinal product in terms of quality characteristics, biological activity, safety and efficacy based on a comprehensive comparability exercise needs to be established.

In principle, the concept of biosimilarity is applicable to any biological medicinal product. However, in practice, the success of developing a biosimilar will depend on the ability to produce a medicinal product which is similar to the reference medicinal product, and to convincingly demonstrate the similar nature of the concerned products. This includes comprehensive physicochemical and biological characterisation and comparison and requires knowledge on how to interpret any differences between a biosimilar and its reference medicinal product." [27]

A special risk factor of biosimilars – as with all biotechnologically produced pharmaceuticals – is their potential immunogenicity with the generation of neutralizing antibodies against the active substance. Pharmaceutical tests can detect only part of the properties of proteins so that safe predictions about immunogenicity are not possible by means of pharmaceutical analyses. Therefore biosimilars are approved under the prerequisite that circumstantial pharmacovigilance studies have to be conducted after their commercial launch to evaluate their safety (including immunogenicity) and efficacy also after a prolonged period of application. (Pharmacovigilance). Post-marketing pharmacovigilance and risk management plans are of extreme importance and those must be strictly followed so that clinical safety of biosimilars can be closely monitored even after their approval [25].

II. Immune System

In order to recognize and protect the human body against foreign substances or invading microorganisms (pathogens), the immune system has developed several defence mechanisms, being the ultimate goal to eliminate these potentially harmful interferences from the body [5].

The Immune system, a very complex system comprising a high amount of cells involved, can be divided in two types of immunity systems: Innate Immunity and Adaptive Immunity (specific immune system).

1. Innate Immune System

Upon exposure to a new pathogen, the body relies on the first line of defence which consists in the innate immunity, a subset of the immune system which is able to recognize and respond immediately in a generic way to the pathogens (non pathogen-specific response). Innate immune responses are dependent on a group of proteins and phagocytic cells that recognize conserved features of pathogens and become quickly activated to help destroying invaders. The downside is: innate immune system does not confer a long-lasting immunity; that is more commonly obtained by the adaptive immune system [28, 29]. However, as adaptive immune response usually takes 4-7 days to start, the innate immune response has a critical role in controlling infections during this period [29].

Microorganisms do occasionally breach the epithelial barricades. It is then up to the innate and adaptive immune systems to recognize and destroy them, without harming the host. Consequently, the immune systems must be able to distinguish self from non-self.

The innate immune system relies on the recognition of particular types of molecules that are common to many pathogens but are absent in the host. These include many types of molecules on microbial surfaces and the double-stranded RNA of some viruses. Pathogen-associated molecules (called PAMPs – *pathogen-associated molecular patterns*) stimulate two types of innate immune responses – Inflammatory responses and phagocytosis by cells such as neutrophils, macrophages, dendritic cells (DC) and natural killer (NK) cells. Both of these responses can occur quickly, even if the host has never been previously exposed to a particular pathogen [28].

The main cell types seen in the initial phase of an inflammatory response are neutrophils, which are recruited into the inflamed, infected tissue in large numbers. Like macrophages, they have surface receptors for common PAMPs and complement, and they are the main cells which engulf and destroy the invading pathogens. The influx of neutrophils is followed shortly by monocytes that rapidly differentiate into macrophages. Thus, Macrophages and neutrophils are also known as inflammatory cells. Later in an infection, the inflammatory responses also involve lymphocytes, which have meanwhile been activated by antigen that has drained from the site of infection via the afferent lymphatics [29].

Upon contact with pathogens, macrophages and NK cells may kill pathogens directly or, in cooperation with dendritic cells, may activate a series of events that both slow the infection

and recruit the more recently evolved intervenient of the human immune system – the adaptive immune system [28-30].

The inflammatory response increases the flow of lymph containing antigen and antigen-bearing cells into lymphoid tissue, while complement fragments on microbial surfaces provide signals that synergize in activating lymphocytes whose receptors bind to specific antigens. Macrophages that have phagocytised pathogens and become activated can also activate T lymphocytes; still, the cells that specialize in presenting antigen to T lymphocytes and initiating adaptive immunity are usually the dendritic cells [29].

2. The Adaptive Immune System

Adaptive immunity is characterized by antigen-specific responses to a foreign antigen or pathogen. A key feature of adaptive immunity is that following the initial contact with antigen, the subsequent antigen exposure leads to more rapid and vigorous immune responses (immunologic memory) [28, 30].

Some cells of the innate immune system directly present microbial antigens to T cells to initiate an adaptive immune response. The cells that do this most efficiently are called dendritic cells, which are present in most vertebrate tissues. They recognize and phagocytise pathogens at an infection site and then engulf pathogens and degrade them intracellularly. Dendritic cells main role however is not to destroy pathogens, but to carry pathogen antigens to peripheral lymphoid organs and then present them to T lymphocytes. As such, they act as antigen-presenting cells (APCs), which directly activate T cells to respond to the pathogen antigens [28, 29].

The adaptive immune system comprises both cellular and humoral immunity, whose principal effectors are, respectively, T cells and B cells.

Adaptive immunity is found only in vertebrates and is based on the generation on T and B lymphocytes of antigen receptors by gene rearrangements, such that individual T or B cells express unique antigen receptors on their surface capable of specifically recognizing diverse antigens of the innumerable infectious agents in the environment. Coupled with fine-tuned specific recognition mechanisms that maintain tolerance (non-reactivity) to self-antigens, T and B lymphocytes bring both specificity and immune memory to vertebrate host defences [5, 28, 30].

Once activated, some of the T cells migrate to the site of infection, where they promote the synthesis and release of soluble factors or mediators (like cytokines) to either kill infected host cells or help other phagocytic cells (mainly macrophages) to destroy the pathogens (cellular immune response). Other activated T cells remain in the lymphoid organ and act as T helper-cells that have an important role in enhancing B cells response to pathogens (humoral immune response). In opposition to cell-mediated immune response, humoral immune response does not have to be developed at the site of infection and, once activated, B cells are able to secrete antibodies that circulate in the body and coat the microbes, targeting them for efficient phagocytosis [5, 28, 30].

Both B and T cells circulate continuously between the blood and lymph. Only if they encounter their specific foreign antigen in a peripheral lymphoid organ they stop migrating, proliferate, and differentiate into effector cells or memory cells [28].

3. Complement System

The complement system consists of more than 15 individual glycoprotein compounds in the plasma that react with each other in a predetermined manner. The result includes the attraction and stimulation of immune cells (e.g. macrophages), the initiation of phagocytosis, or the lysis of cells that are targeted by the complement system [5, 28].

Although the complement system is usually associated with humoral immune response, by its ability to amplify and "complement" the action of antibodies, there are some components of complement that can also be characterized as *pattern recognition receptors* (PRRs) that can be activated directly upon recognition of *pathogen-associated molecular patterns* (PAMPs) [28].

Recognition of pathogen PAMPs leads to activation/production of the complement cascade, cytokines, and antimicrobial peptides as effector molecules. In addition, pathogen PAMPs as host danger signal molecules activate dendritic cells to mature and to express molecules on dendritic cell surface that optimize antigen presentation to respond to foreign antigens [30].

The early complement components are activated first and they all act locally to activate C3, which is the pivotal component of complement. The larger fragment of C3, called C3b, binds covalently to the surface of the pathogen and, once in place, not only acts as a protease to catalyze the subsequent steps in the complement cascade, but also is recognized by specific receptors on phagocytic cells which enhance the ability of these cells to phagocytose the pathogen. The smaller fragment of C3 (called C3a), as well as fragments of C4 and C5, act independently as diffusible signals to promote an inflammatory response by recruiting phagocytes and lymphocytes to the site of infection.

The phagocytic cells use a combination of degradative enzymes, antimicrobial peptides, and reactive oxygen species to kill the invading microorganisms and, in addition, release signalling molecules that trigger an inflammatory response and begin to assemble the forces of the adaptive immune system [28].

III. Antibodies

Following the success of recombinant proteins, such as insulin, therapeutic Monoclonal Antibodies (mAbs) today represent the second wave of innovation created by the biotechnology industry in the past 20 years [5].

1. Main basis

Upon the presence of foreign molecules in the body, the immune system is capable of generating its own physiological antibodies to recognize and eliminate/inactivate invading material with antigenic determinants. All antibodies are immunoglobulins [31] and are glycoproteins produced by B lymphocytes (plasma cells). As part of the specific humoral immune response, they are secreted into the blood or lymph system and neutralize foreign invading microorganisms (bacteria, parasites or viruses) or other non-endogenous substances.

Antibodies are strictly defined products that contain a complementary region towards the binding part of the antigen (epitope) [2, 5].

2. Basic Structure and Functions

Though differently produced, basic structure of antibodies is most of the times the same.

Antibodies are ~150kDa immunoglobulins (IgGs), usually represented as Y-shaped, and composed by four polypeptide chains, two identical copies of a heavy chain (H) and two identical copies of a light chain (L) [32-35]. Each light chain is bound to a heavy chain by a disulfide bond and by such noncovalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds, to form a heterodimer (H-L). Similar noncovalent interactions and disulfide bonds link the two identical heavy and light (H-L) chain combinations to each other to form basic four-chain $(H\text{-}L)_2$ antibody structure, a dimer of dimers [35].

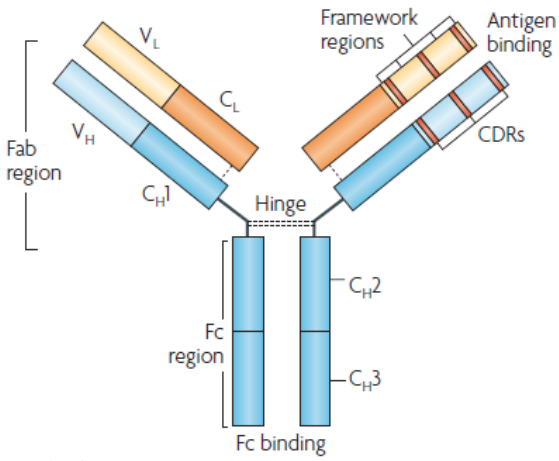

Figure 2 – Antibody Basic Structure.
In Hansel, Kropshofer et al. 2010.

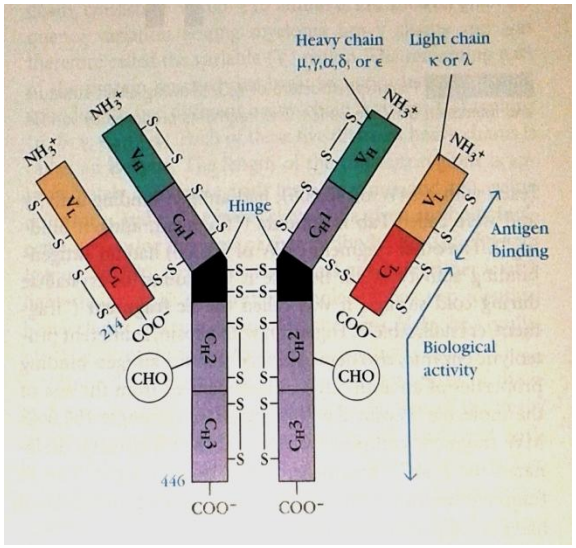

Figure 3 – Schematic diagram of antibody structure.
In Goldsby, Richard et al. 2003.

The heavy chains contain a variable domain (V_H) and three constant domains (C_H) whereas the light chains contain a variable domain (V_L) linked to a single constant domain (C_L) [4, 33].

Variable regions (V_L & V_H) are, by excellence, the antigen-binding site and these are located in Y's arms, also called antibody's Fab (fragment, antigen binding) region.

Within the variable chains, biding-site is defined by highly variable amino acid sequences – the *complementarity-determining regions* (CDRs) – and these are the major site of interaction with an antigen. There are three CDRs (CDR1, CDR2 and CDR3) on each variable chain and antibodies diversity is achieved by variations in these CDRs aminoacid sequences [32, 34, 35].

Although a highly selective antigen binding is a signature and functionally crucial property of antibodies, when considering the role of antibodies in defence against disease, it is important to note that antibodies generally to not kill or remove the pathogens just by binding to them. Apart from binding to pathogens, antibodies also need to invoke responses that will result in the removal of the antigen. These responses are achieved by interactions of the heavy-chain constant region (C_H) with other proteins or cells. The base of the base of the Y is, thus, the region that determines effector functions of antibodies, by modulating immune cell activity. This region is called Fc (Fragment, crystallisable) and its role is to ensure that each antibody generates an appropriate immune response for a given antigen. This is, most of the times, dependent on interaction of the Fc region with other proteins: FcγRs for antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), complement components for complement-dependent cytotoxicity (CDC) and FcRn for long serum persistence (FcRn is structurally related to MHC class I molecules and protects IgG from degradation, resulting in long serum half-life). Different isotypes of immunoglobulins [please refer to further section "*Immunoglobulins – Antibody Classes and Biological Activities*"] are defined by the structures of immunoglobulin Fc domains. [32-37].

1. Antibody-Mediated Effector Functions

Antibodies operate through various mechanisms (Figure 4). These can be divided in Fab-dependent actions and Fc-dependent effector functions.

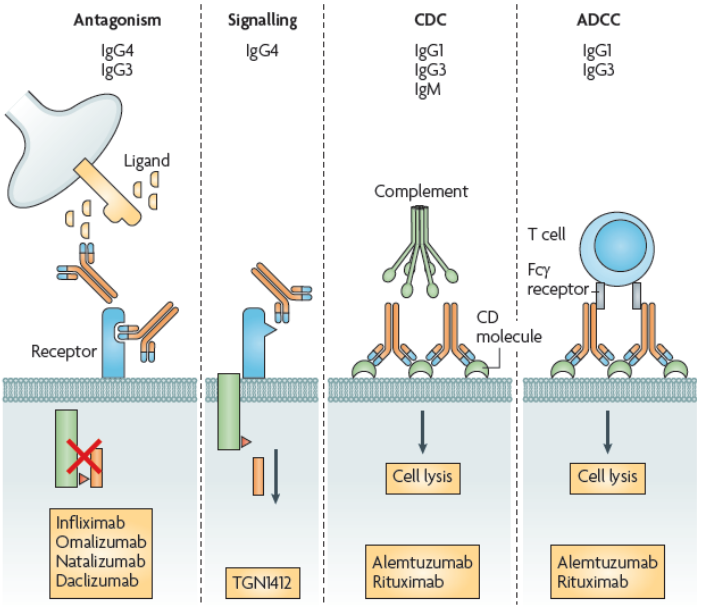

Figure 4 – Functions of mAbs. ***In Hansel, Kropshofer et al. 2010.***

As mechanisms controlled by Fab region, the following can be identified:

(i) Antagonism: Fab region binds to the antigen and blocks its interaction with the ligand

(ii) Signalling: Signal transduction inducing by binding of the antibody to a receptor.

Fc portion, on another hand, is responsible for antibody-mediated effector functions and these can include:

(i) Antibody-dependent cell-mediated cytotoxicity (ADCC)

ADCC occurs when antibodies bind to antigens on target cell (virus infected cell from the host or tumour cell) and the antibody Fc domains engage Fc receptors (FcγRs) on the surface of immune effector cells such as neutrophils, macrophages and natural killer cells. The resulting complex triggers a cytolytic response to induce apoptosis, phagocytosis or lysis, depending on the type of mediating effector cell (figure 4). In ADCC, the antibody acts as a newly acquired receptor enabling the attaching cell to recognize and kill the target cell [5, 35, 38, 39].

(ii) Complement-dependent cytotoxicity (CDC)

Another effector function/ mode of action of antibodies is the so called "complement-dependent cytotoxicity (CDC)". Activation of the complement system can lead to lysis of the antigen-presenting cell or can induce inflammation reactions aimed to eliminate these cells as soon as possible.

A prerequisite first step for CDC is the recruitment of the glycoprotein C1q of the complement system that will bind to the Fc portion of the antibody. This triggers a proteolytic cascade to activate complement. This can lead to the production of the glycoprotein C3b as well as to the formation of a membrane attack complex (MAC) that kills the target cell by disrupting its cell membrane. Alternatively, tumour-cell-bound C1q can bind to complement receptors, such as C1qR, CR1 (CD35) and CR3 (CD11b/CD18), on effector cells, such as neutrophils, macrophages and natural killer cells. This can trigger cell-mediated tumour-cell lysis or phagocytosis, depending on the type of effector cell (figure 5) [5, 35, 38].

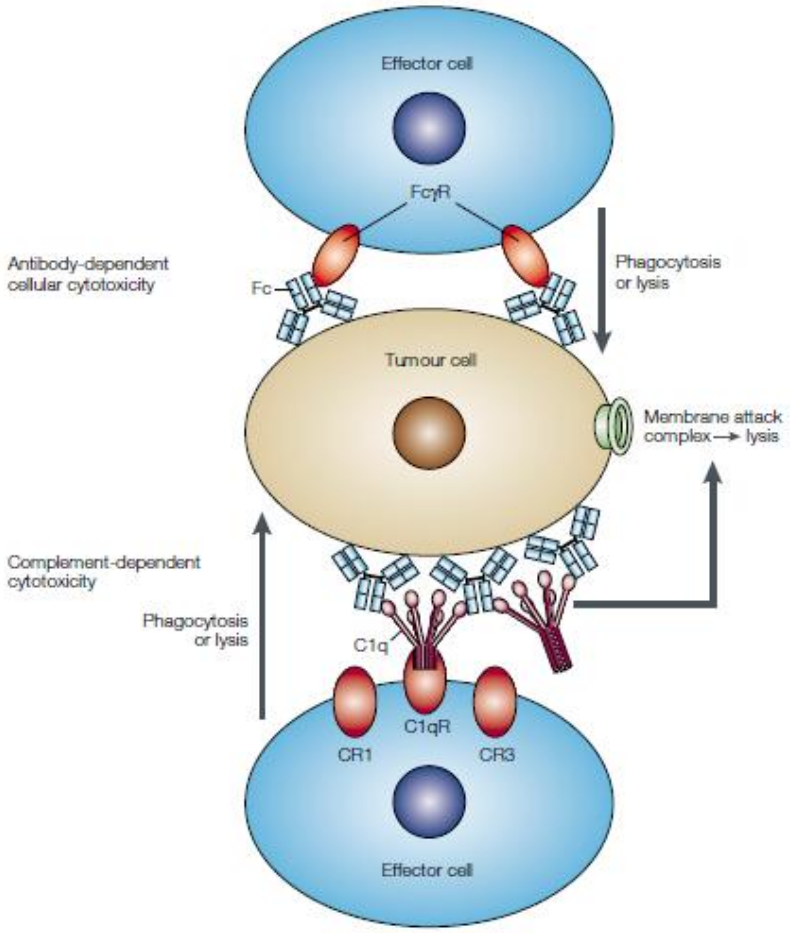

Figure 5 – Antibody effector functions. In Carter, P. 2001.

(iii) Antibody-dependent cellular phagocytosis

The promotion of phagocytosis of antigens by macrophages and neutrophils is an important factor in antibacterial defences. Antibodies' Fc constant region can bind to Fc Receptors present on the surface of macrophages and neutrophils. The binding of Fc receptors with several antibody molecules complexed with the same target such as bacterial cell, produces an interaction that results in the binding of the pathogen to the phagocyte membrane. Inside the phagocyte, the pathogen becomes the target of various destructive processes that include enzymatic digestion, oxidative damage and membrane-disrupting effects of antibacterial peptides [35].

2. Immunoglobulins – Antibody Classes and Biological Functions

Antibodies can be classified according to GADME system based on their configuration and function. The five different classes (or isotypes) are Immunoglobulins G, A, D, M and E and an overview of each one's specificity is covered in Table 1 [5, 35, 36].

Table 2 – Immunoglobulins Classes and Biological Functions

Class	Sub-Class	Function	Molecular Mass [KDa]	Rate of carbohydrates	Proportion of total Ig [%]	$t_{1/2}$ [days]
IgG	IgG 1	Main Ig in blood and extravascular region. Binds to antigen and toxins.	150	2%	75	21
	IgG 2					21
	IgG 3					7
	IgG 4					21
IgA	IgA 1	Main Ig in secretions. IgA is specialized in the defence against antigens on the surface of mucosal membranes, like intestine and nose.	Monomer: 160 Dimer: 390 (most abundant form) Secretory dimer: 385	10%	15	6
	IgA 2					6
IgD	IgD	Mainly in humans. Found on B lymphocytes during some stages of maturation and is jointly responsible for their activation.	180	11%	0,5	3
IgM	IgM	First Ig to be produced by immune system upon the present of an unknown antigen. After the primary reaction its concentration decreases, in opposition to the increase of IgG. Usually presented in pentameric form. IgM favours agglutination and cytolysis.	970	10-12%	7	10
IgE	IgE	Derived from adenoid tissue and then transported into the blood. Although IgE has a low serum concentration, it has a main role in allergies, being responsible for about 90% allergic reactions.	190	12%	0,002	2

Immunoglobulin G (IgG) represents the most important class of immunoglobulins with a serum concentration of 12mg/mL, the lowest molecular mass of 150kDa and the longest half-life of 21 Days (with the exception of the sub-class IgG3 which only has a 7Days half-life). Due to differences in chemical structure (differences in Fc portion and disulfide bonds) and biological

function, four sub-classes of IgGs can be identified: IgG1, IgG2, IgG3 and IgG4, being the IgG1 the most abundant [5].

Following the first exposure of an individual to an unknown antigen, the first occurrence of specific IgG can be detected in average 3 weeks later. Upon the following exposures to the same antigen, however, the production of IgG is much quicker and in larger amounts. This is characterized as immune memory, which confers the immune system the ability to mount rapid recall immune response [5, 30].

IgG class is the only class of immunoglobulins capable of crossing the placental barrier and its transference from the mother is responsible for the initial immunity of the foetus during the first months of pregnancy [5].

In general, immunoglobulins – as proteins with hydrophilic and glycosylated moieties – have a high molecular weight of approximately 150-200kDa. The only exception is the tentameric IgM which has a molecular weight of 970kDa [5].

1. Antibodies glycosylation

Antibodies are glycoproteins and almost all antibodies have the sites of attachment for carbohydrates stricted to the constant region. Although it is not completely understood the role of glycosylation of antibodies, it is generally observed that the oligosaccharide present in glycoproteins contributes to their solubility and stability.

Changes in glycosylation (or its absence) has an impact in the rate of antibodies clearance from the serum as well as in the efficiency of interaction between antibodies and complement system and between antibodies and Fc receptors. Antibody glycosylation is a common post-translational modification and the use of glycoengineering to produce antibodies with specific glycoforms may be required to achieve the desired therapeutic efficacy. [31, 35, 40, 41].

2. Monoclonal Antibodies (mAbs)

There are two types of antibodies: polyclonal and monoclonal.

Multiple antibodies produced against the immediate offending antigen, as well as antibodies produced against previously encountered antigens owing to the stored memory of past called anamnestic reaction, can be considered polyclonal antibodies [2].

The use of vaccines for immunization is an excellent example of preventive use of Abs [42].

The development of new techniques in biotechnology field enabled Abs usage to be extended to treatment and, biotechnology-synthesized Abs against a particular antigen [43], become known as monoclonal antibodies [2, 3].

By definition, mAbs are biological medicines produced using techniques of immunology and genetic engineering. Their first appearance was in 1975 when the Nobel Prize winners Köhler and Milstein published their seminal manuscript on hybridoma technology enabling the production of mouse monoclonal antibodies [4, 11, 32, 34, 44, 45]. Back then, mAbs were

produced by injecting laboratory mice with a specific pathogen. This antigenic exposure in the mouse would then result in the development of specifically sensitized B cells which were able to produce specific antibodies. The genetic machinery of these sensitized B cells would then be extracted and inserted into a myeloma B cell thus forming a hybridoma with immortalised B cells, capable of producing a higher amount of monospecific and homogeneous antibodies. These precise antibodies were called "monoclonal antibodies" since they were derived from a single line or clone of specifically sensitized, genetically engineered B cells [2-4, 32, 46].

2.1. Types of mAbs

Although mouse-derived mAbs were considered a landmark step in drugs investigation, polyclonal as well as monoclonal antibodies when produced in an ordinary animal had very limited use in human beings, because those contained some of the unwanted animals antigens which would in turn provoke immune reactions in humans [2, 32, 47]. "HAMA response" (**H**uman **A**nti-**M**ouse **A**ntibodies response) is an example of those human immune reactions which can quickly result in the human elimination of the mouse-derived proteins, reducing therefore its efficacy [44].

The induction of HAMA responses has been postulated to be a major impediment to the success of mAb therapy. There are two types of HAMA responses: antiisotypic and anti-idiotypic. Hence, the development of HAMA has two drawbacks: first, retreatment may result in anaphylaxis and allergy; and, second, retreatment may be less effective than previous treatments [32].

In order to prevent these immune reactions, further research was done and, together with some technical advances, it became possible the transition from mouse – via chimeric and humanised – to fully human mAbs, with reduced potentially immunogenic mouse components [4, 38].

In summary:

First Generation Antibodies – Murine, Rat or Rabbit proteins derived by hybridoma technology, following immunization of the animal with an antigen preparation [3, 4, 38].

Second Generation Antibodies – To overcome the obstacles of first generation antibodies, Winter *et al.* pioneered the techniques to humanize mAbs, by removing the reactions that many earlier mAbs caused in some patients [3, 47]. Nowadays, DNA technology or genetic engineering is used to construct hybrids composed of human antibody regions linked with a murine or primate backbone. These are labelled as second generation mAbs and are referred to as chimeric, humanised or human mAbs [3].

(i) ***Chimeric Antibodies*** – These are composite of antibodies from two different species and are obtained by joining the antigen-biding parts (variable domains) of a mouse monoclonal antibody with the effector parts (constant domains) of a human antibody [3, 4, 38].

(ii) ***Humanised Antibodies*** – Human antibody containing the complementarity-determining regions (CDRs) from a non-human source. These antibodies combine only the amino acids responsible for making the antigen-binding site (the hypervariable region) of a mouse antibody, remaining the rest of antibody as a human molecule – in summary, only the human antibody hypervariable regions are replaced [3, 4, 38].

(iii) ***Human Antibodies*** – can be produced by the following techniques:
(a) ***Recombinant DNA technology*****:** By inserting random human genes coding for variable Fab portion of human antibodies into the genome of filamentous bacteriophages. As the bacteriophage replicate they display antibody Fab portion on their surface. The bacteriophages are subsequently mixed with an antigen to select those producing complementary Fab portions. Those bacteriophage genomes are then converted into plasmids that can subsequently produce specific Fabs in bacteria.

(b) ***Transgenic mice*****:** Transgenic technology has been exploited to make a transgenic mice that have human Ab gene loci inserted into their bodies (using embryo stem cell method) and their own genes for making antibodies 'knocked out'. Therefore, mouse can be immunized with the desired antigen and produces human Abs.

(c) ***Phage display*****:** This is another technique human mAbs production. It is used when mAbs do not directly recognize antigen or when antigen is undetectable normally and expressed only in disease [3].

These fully humanised mAbs were initiated during the early 1990s by Lonberg and his group, who managed to inactivate the key genes which produce antibodies in mice and instead transplanted the human antibody genes.

The advantages of unlimited supplies of all human specific monoclonal antibodies for therapeutic – such as improvement of success rate and decrease of immunogenic potential – are promising and might fulfil Ehrlich's original notion of "magic bullet" [2-4, 32, 44, 46, 48]

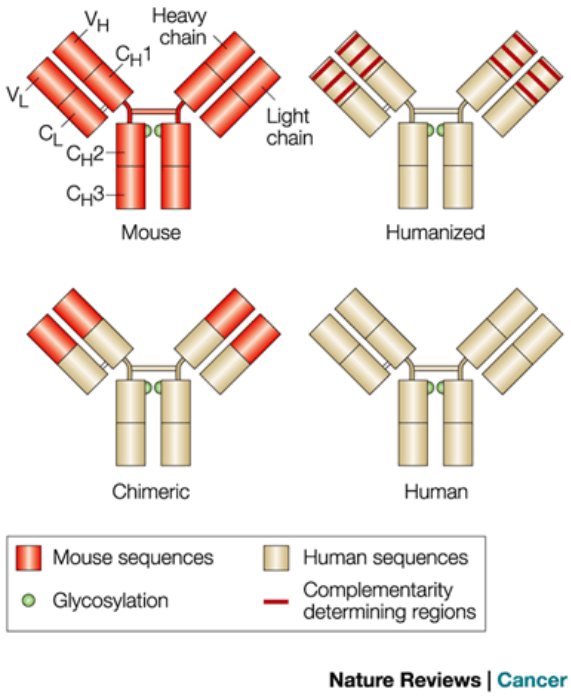

Figure 6 – Differences between differently-produced mAbs. *In Paul Carter, 2001*.

Curiosity: The United States adopted name (USAN) Council has outlined specific guidelines for the nomenclature of mAbs. These guidelines provide a foundation of knowledge about a specific mAb just by looking at the generic name. Below are described some basics of these guidelines, as well as some examples of correspondent mAbs [3, 4, 49]:

1. All mAbs have the suffix "mab" as identifier of the class of medicine.

2. The infix preceding "mab" usually indicates the source of antibody:
- ***a.*** Letter "**o**" for entirely **murine** antibodies – *e.g. muromonab*
- **b.** Letters "**xi**" for **chimeric** antibodies – *e.g. rituximab*
- **c.** Letters "**zu**" for **humanised** antibodies – *e.g. alemtuzumab*
- ***d.*** Letter "**u**" for **human** antibodies – *e.g. adalimumab*

3. The infix preceding the source of the antibody refers to medicine target:

INFIX	**DEFINITION**	***EXAMPLE***
tu / t	tumours	*trastuzumab*
li / l	immunomodulator	*adalimumab*
ba / b	bacterial	
ci / c	cardiovascular	
fu / f	antifungal	
ki / k	interleukins	*canakinumab*
ne / n	neurons as targets	
so / s	bone	*sulesomab*
vi / v	viruses, antiviral indications	*palivizumab*

4. The starting prefix is a distinct syllable carrying no special meaning and unique for each drug.
5. If the product is radiolabeled or conjugated to another chemical such as a toxin, a separate word is used to identify the conjugate.

2.2. Therapeutic advances

Since 1975, when Kohler and Milstein developed a procedure to efficiently produce monoclonal antibodies (mAbs) it has been widely believed that these molecules would be ideal reagents for imaging and therapy, similar to the "magic bullets" idealized by Paul Ehrlich at the beginning of the 20th century.

However, following the first-reaction of excitement, it rapidly became clear that, although highly specific and with potential to enable a tailored therapy, these molecules would still need improvements in the serious problems they were facing. As the first mAbs were murine molecules, they were usually recognized as foreign, leading to their elimination by the patient's immune system. Moreover, as antibodies often needed to interact with certain elements of the immune system to be effective, their murine nature would also prevent a proper interaction with components of the human immune system, compromising this way their biological efficacy.

Another major improvement came with the development of in vitro selection methods, the most successful one being phage display. With the ever increasing power of antibody engineering, it became possible to clone entire repertoires of antibody fragment genes, from immunized or nonimmunized animals, including humans.

The creation of chimeric, humanised or fully human antibodies was a major breakthrough and led to a wave of new commercialized antibodies. However, mAb-based treatments face several other limitations, which limit their widespread use as therapeutics – not only production costs are much higher leading, in turn, to higher commercialization costs which limit patients' access; but also mAbs specificities (like molecular size and mode of action) may limit penetration of the molecule within the body as well as limit the administration mode to others than oral administration.

Antibody engineering has played a major role in the development of the first generation of therapeutic antibodies. It is now being used in several ways to obtain a new generation of optimized antibodies with a modified Fc region capable of circumventing some of the limitations described before. However, the potential offered by antibody engineering can go further than optimization and it is a way to create entirely new Ig domain-based molecules, not found in nature, which can be tailored to match desired characteristics.

Improved delivery; greater selectivity for targets; reduced dosing; enhanced penetration within body barriers and possibility of oral administration are some of the challenges and future opportunities faced by the therapeutic mAbs field [33, 50].

2.3. Raising concerns following mAbs introduction into the market

Among the advantages of protein therapeutics, such as mAbs, when compared with low-molecular-mass drugs, the top ones are their specificities – which facilitate precise action – and their long half-lives – which allows infrequent dosing.

mAbs are having a great success rate in clinic, not only in clinical trials, which have currently hundreds of them being tested [4], but also in market approval rate which is greater than for chemicals, 20% to 5%, respectively [4, 51]. Taking European case for example, according to data published in European Medicines Agency (EMA)l website, in 15 years of investigation (1996-2011) 39 marketing authorization applications were submitted to EMA. 35 of those were approved and, in the mean time, 7 were withdrawn, which means that, in total, 28 of those mAbs are still in European market.

Despite all known advantages, biopharmaceuticals discovery also brought to scientific community and regulatory authorities some challenges until then unknown. Due to biopharmaceuticals characteristics in what concerns size, complexity and manufacture, these molecules promptly show to require different approaches for efficacy, effectiveness, safety and toxicology assessment and approval by scientific community and regulatory agencies [4, 8, 9, 51, 52].

One of the main differences between biopharmaceuticals and chemical molecules is their mechanism of action within the body. mAbs have more precise actions since their manufacture process is fine-tuned for specific therapeutic actions [4, 32]. If, from one point of view, this is clearly an advantage of these medicines, it may also represent a great factor to have in account for the evaluation and prediction of mAbs' side effects.

Studies had shown that numerous mAbs side effects may be related to their specific targets [4], meaning, being related to their mechanism of action. The goal of this project is to infer the actual correlation between mAbs' reported adverse events and mAbs' specific mechanism of action.

THESIS OBJECTIVES

The goal of this project was to correlate mAbs' adverse events with their specific mechanism of action, aiming to prove that one influences the other. By proving that one influences the other, it was hypothesised that mAbs mechanism of action may help to predict their adverse events.

This project had one objective, which was then dived in four tasks, to achieve one milestone:

- ✓ **1st Objective** – Correlation of mAbs' mechanism of action with their reported adverse events:
  - **Task I** – Collection of available and relevant information regarding mAbs advances since their first appearance in drugs' market (EMA and FDA data collection).
  - **Task II** – Collection of available and relevant information regarding mAbs authorised in Europe, including withdraws since first approval.
  - **Task III** – Sorting of mAbs authorised in Europe by classes with similar mechanisms of action.
  - **Task IV** – Comparison of adverse events reported within each class.

  Milestone: mAbs' adverse events are closely related to mechanism of action.

It is important to note that for the purpose of Task IV, the Adverse Events which were compared comprised:

- Adverse Events as per the Initial European Marketing Authorisation Documents

  (Source: mAbs' first EPARs, available in EMA website [53])
- Adverse Events reported in Europe following Marketing Authorisation

  (Source: European database of suspected adverse drug reaction reports [54])
- Adverse Events reported in the United States following Marketing Authorisation

  (Source: DrugCite.com website [55])

The reason for including the Adverse Events as per the Initial Documents for European Marketing Authorisation in addition to the Adverse Events reported post-Marketing Authorisations (MA) was for the comparison between the information firstly published on each mAb (Initial Documents for EU MA are mainly based on clinical trials data) and the information acquired on post-MA period (real life data). It was interesting to see the differences in Adverse Event reported pre and post MA.

METHODOLOGY

I. Research sources

All data collected, reviewed, analysed, summarized and compared has been obtained mainly through online research, including not only Pubmed website – for applicable articles, reviews and upcoming concerns and discoveries in mAbs mechanism of action field – and EMA website – where all European Public Assessment Reports (EPARs) and Summaries of Product Characteristics (SmPC) for all mAbs authorised in Europe were available [53] – but also other applicable databases, as:

a) DrugBank Open Data Drug & Drug Target Database [56], for information about mechanism of action and drugs' targets;

b) ATC/DDD Index 2012 [57], for division of active substances into different groups according to the organ or system on which they act and their therapeutic, pharmacological and chemical properties;

c) DrugCite.com website [55], as public access database of adverse events reported through the FDA Adverse Events Reporting System (FAERS);

d) European database of suspected adverse drug reaction reports [54], as public access database created in 2012 for information on suspected side effects for approved drugs.

All efforts were made to keep online search compliant with MeSH Terminology [58] whenever applicable. For Adverse Events listing, Medical Dictionary for Regulatory Activities (MedDRA 17.1) was used.

Some books, magazines, reports and presentations were also sources for data collection.

II. Data Treatment

For the comparison of adverse events reported within each class of mAbs the data collected and compared has been the European (EU) and United States (U.S.) data, which, in turn, was made available through different databases, with different data sources, formats and classifications (Table 2).

Table 3 – Differences in presentation of data pertaining to Adverse Events (AEs) reported in EU and in the U.S.

Data characteristics	EU database of suspected adverse drug reaction reports [54]	DrugCite.com website [55]
Source	**EudraVigilance** - Comprises submissions by national medicines regulatory authorities and pharmaceutical companies that hold marketing authorisations for the medicines	FDA Adverse Events Reporting System (**FAERS**) - Includes adverse events if the drug is flagged as a suspect drug causing the adverse event.

Data characteristics	**EU database of suspected adverse drug reaction reports [54]**	**DrugCite.com website [55]**
Updating frequency	**cases reported up to the end of the previous month** (updates are done on the 15th of the month)	uncertain (information not available) - at the time of data collection, only had data from Q1 2004 **until Q3 2012**
Data format	chart format	chart format
Charts data sorting	**always the same sorting layout**, not dependent on values	**descending values**, variable sorting layout dependent on values
Charts data labels available	only displayed in the online format (for the purpose of this project, those were retrieved one by one)	yes
Charts data classifications	number of **individual cases by reaction groups** – MedDRA System Organ Classes (SOC) The reaction groups are based on a classification of the side effect (also known as adverse drug reaction), using the MedDRA dictionary of terms.	top **categories of adverse events** – MedDRA High-Level Group Terms (HLGT)
% of the AE to all AEs reported for the drug	no	yes - Percent does not represent a rate related to drug utilization

Since the data retrieved from both EU and U.S. databases was significantly different, some treatment of data was necessary for enabling the comparison of EU and U.S. data.

For each mAb, it was firstly analysed the EU and U.S. raw data and only then, a cumulative data chart was created. For that cumulative data chart to be created, some treatment of data was performed:

1. U.S. data classification was re-arranged and grouped into a classification similar to EU data classification: MedDRA System Organ Class (SOC) system
2. only MedDRA SOC totals were displayed in the cumulative chart
3. only absolute values of reported adverse events were displayed in the cumulative chart

RESULTS AND DISCUSSION

At an initial stage, no mAbs were excluded from research, as the purpose was the acquisition of global knowledge. Only then the search started to be redefined according with this project's focus: in this case, mAbs authorised in Europe.

Based on dates of mAbs' marketing authorizations by EMA, it was decided to review data from 15 years of research, from 1996 to 2011, which corresponds to 35 mAbs.

1996 year was the first time mAbs were authorised by EMA: Arcitumomab; Igovomab and Anti-melanoma mab fragments. Although all those mAbs have already been withdrawn since then, they were still an important milestone in the history of biopharmaceuticals in Europe.

2011 year, on another hand, was also a year for three mAbs authorizations granted by EMA: Belimumab; Denosumab and Ipilimumab. Those, in opposition to 1996's mAbs, are still authorised for marketing.

As the main goal of this project is to correlate mAbs with their mechanism of action, it was considered important to classify and sort in classes the 35 mAbs approved in Europe from 1996 to 2011. mAbs' classification was of extreme importance for the project because all subsequent comparisons would be made according with grouping done at this stage. For that reason, sorting had to be performed in a consistent way for all mAbs to be analysed and the method chosen was to classify mAbs by their targets.

References used for these classification and sorting process included not only EPARs/ SmPC for all 35 mAbs authorised in Europe [53], but also other applicable online databases that also classify medicines: i) DrugBank Open Data Drug & Drug Target Database [56] which provides information of mechanism of action and drugs' targets, and ii) ATC/DDD Index 2012 [57], which divides active substances into different groups according to the organ or system on which they act and their therapeutic, pharmacological and chemical properties. In the ATC database, drugs are classified in groups at five different levels: (a) 1st level divides drugs into fourteen main groups; (b) 2nd level divides by pharmacological/therapeutic subgroups; (c) 3rd and 4th levels dives by chemical, pharmacological and therapeutic subgroups and (d) 5th level corresponds to the chemical substance.

I. Classification of mAbs

Classification and sorting of mAbs based on their targets, resulted in Table 2, below presented:

Table 4 – Classification of European authorised mAbs by target [56, 57]

Trade name	International Non-proprietary Name	Target	ATC classification
Removab	Catumaxomab	CD3 + EpCAM	Antineoplastic
Yervoy	Ipilimumab	CTLA-4	
Zevalin	Ibritumomab Tiuxetan	CD20	
Arzerra	Ofatumumab		
MabThera	Rituximab		
MabCampath	Alemtuzumab	CD52	
Erbitux	Cetuximab	EGFR	
Vectibix	Panitumumab		
Herceptin	Trastuzumab	HER-2	
Mylotarg	Gemtuzumab ozogamicin	CD33	
Avastin	Bevacizumab	VEGF	
Lucentis	Ranibizumab		
Synagis	Palivizumab	Fusion glycoprotein	Anti-infectives
Mycograb	Efungumab	Hsp90	
Xolair	Omalizumab	human IgE	Drugs for obstructive airway diseases
Soliris	Eculizumab	Complement C5	Immunosuppressants
Tysabri	Natalizumab	Integrin α4β1	
Raptiva	Efalizumab	Integrin LFA-1	
Simulect	Basiliximab	Interleukin-2 receptor	
Zenapax	Daclizumab	Interleukin-2 receptor	
Benlysta	Belimumab	BLyS	
Ilaris	Canakinumab	Interleukin-1 beta	
Stelara	Ustekinumab	IL-12/23p40	
RoActemra	Tocilizumab	Interleukine-6	
Trudexa	Adalimumab	TNFα	
Remicade	Infliximab		
Cimzia	Certolizumab pegol		
Simponi	Golimumab		
LeukoScan	Sulesomab	NCA-90	Diagnostic agents [43, 59]
Scintimun	Besilesomab	NCA-95	
CEA-Scan	Arcitumomab	CEA	
HumaSPECT	Votumumab	CTAA16.88	
Xgeva	Denosumab	RANKL	Drugs for treatment of bone diseases
ReoPro	Abciximab	αIIbβ3	Anti-thrombotic agents

It is here to be investigated if similar mechanisms of action result in similar reported adverse events and, for that, the classes previously established are the basis for all comparisons to be performed. Comparisons will be made first within mAbs of the same class, and secondly across different classes are important as well, so that conclusions can be made on which adverse events can be expected for a specific mechanism of action.

If everything goes as expected, European authorised mAbs adverse events will be able to be correlated with their mechanism of action and same class mAbs should have similar reported adverse events.

1. Selection of 3 Classes of mAbs to be analysed

Due to the extensive number of mAbs available in Europe, it was decided to study only 3 classes. The selected classes are the ones below described

Table 5 – Classification of European authorised mAbs by target– restricted to the 3 classes to be analysed

Trade name	International Non-proprietary Name	Target	ATC classification
Zevalin	Ibritumomab Tiuxetan	CD20	Antineoplastic
Arzerra	Ofatumumab		
MabThera/Rituxan	Rituximab		
Avastin	Bevacizumab	VEGF	
Lucentis	Ranibizumab		
Trudexa	Adalimumab	TNFα	Immunosuppressants
Remicade	Infliximab		
Cimzia	Certolizumab Pegol		
Simponi	Golimumab		

I. Anti-TNF mAbs

TNFα activity was first detected over 100 years ago when physicians noted that some cancer patients experienced shrinkage of their tumours if they also had a serious bacterial infection. In recent years, however, it has been learned that TNFα has a wide range of biological activities in addition to its ability to kill tumour cells. For example, TNFα has been shown to induce mitogenesis of fibroblasts, induce expression of adhesion molecules on endothelial cells, stimulate interleukin-1 and prostaglandin E2 synthesis in macrophages, trigger a respiratory burst and degranulation in neutrophils, increase hepatic synthesis of acute phase proteins, suppress collagen synthesis by fibroblasts and act on the hypothalamus to induce fever.

These widely varied activities, some of which play a role in immune reactions, some in inflammatory reactions, and some in pathophysiological reactions, attest to the complexity of this cytokine. [60-64].

TNFα is a pluripotent cytokine secreted by macrophages in response to a variety of inflammatory agents.

Gram-negative lipopolysaccharide (LPS) has been extensively studied as a stimuli for macrophage activation [61, 65]. Lipopolysaccharide (LPS) is a glycolipid that constitutes the major portion of the outermost membrane of Gram-negative bacteria. It is also referred in literature as Endotoxin, and this term was introduced in the 19th century to describe the component of gram-negative bacteria responsible for the pathophysiological phenomena associated with gram-negative infections [61, 65, 66].

LPS comprises three parts: O antigen; core oligosaccharide and Lipid A. *It is now established that the lipid A moiety possesses most of the biological activities of LPS and the interaction of the lipid A moiety of LPS with macrophages results in the rapid induction of the syntheses of pro-inflammatory molecules, like tumour necrosis factor (TNF), interleukin 1 (IL 1), and other proteins [65, 66].*

Tumour necrosis factor-alpha (TNFα) is one of several cytokines referred to as pro-inflammatory and it is a vital component of the inflammatory process [44, 64, 67]

The precursor form of TNFα, transmembrane TNFα (mTNF), is expressed on the cell surface of activated macrophages and lymphocytes as well as other cell types (endothelium). Membrane-bound mTNF is subsequently cleaved by a metalloproteinase, TNFα converting enzyme (TACE), which releases the secreted soluble form of TNFα, a 17kD polypeptide [67, 68].

Most cellular responses to TNFα are triggered through its interaction with two structurally distinct receptors: type I (TNFR1 – 55kd) and type II (TNFR2 – 75kd) [44, 61, 62, 64, 67]. Both receptors are transmembrane glycoproteins with multiple cysteine-rich repeats in the extracellular N-terminal domains. Extracellular domains of TNFα receptors are very similar in

structure and function, while intracellular domains of TNFα receptors are distinct and transduce their signals through both overlapping and distinct pathways [67].

While binding of TNFα to TNFR1 seems to induce cytotoxicity, fibroblast proliferation, synthesis of prostaglandins, up regulation of adhesion molecules and NF-KB activation; the role of TNFR2 is less well defined, but it appears to concentrate soluble TNFα at the cell surface for transfer to TNFR1 [69].

Secreted TNFα binds to both TNFR1 and TNFR2, while the membrane-bound TNFα binds mainly to TNFR2. Like TNFα, these receptors exist both as a membrane-anchored form, where they mediate the pleiotropic pathophysiological effects of TNFα, and in a soluble form, where they can bind and neutralize bioactive TNFα [67].

A wide range of biological good outcomes have been associated to TNFα. Nonetheless TNFα has also been implicated as a major mediator of septic shock and cachexia associated with chronic disease states. A possible reason for the different outcomes associated to TNFα might reside in the quantitative rather than qualitative aspects of TNFα physiology: while "appropriate" amounts of TNFα are mainly protective to the host, an excess of TNFα has been linked to numerous disease states. A qualitative explanation might be that the cell-bound rather than secreted version of TNF is advantageous to the host [67, 70].

Elevated levels of TNF are found in the synovial fluid of rheumatoid arthritis patients and play an important role in both the pathologic inflammation and the joint destruction that are the hallmarks of rheumatoid arthritis [63, 71].

As a key early mediator of the inflammatory process, TNFα has become a major target of numerous pharmaceutical investigations, which have yielded several unique proteins that bind and neutralize TNFα bioactivity. In theory, specific monoclonal antibodies anti-TNF may establish connections with TNF receptors on cellular surface and inhibit, this way, T cells and macrophages functions [44, 67].

Because of their relative novelty and recent development, little is known about the long-term consequences of anti-TNFα biopharmaceuticals or what effects they might have at the cellular level [67].

1. mAbs targeted for TNF

Table 6 – anti-TNFα mAbs approved indications for Europe and for the United States

Anti-TNFα mAbs								
ADALIMUMAB		INFLIXIMAB		GOLIMUMAB		CERTOLIZUMAB PEGOL		Indications
U.S.	EU	U.S.	EU	U.S.	EU	U.S.	EU	
X	X	X	X	X	X	X	X	*Rheumatoid Arthritis (RA)*
X	X							*Polyarticular Juvenile Idiopathic Arthritis*
	X						X	*Axial spondyloarthritis*
X	X	X	X	X	X		X	*Ankylosing Spondylitis*
X	X	X	X	X	X		X	*Psoriatic Arthritis*
X	X	X	X					*Psoriasis*
X	X	X	X			X		*Crohn's Disease*
X	X	X	X	X	X			*Ulcerative Colitis*

1.1. ADALIMUMAB

The active substance ADALIMUMAB is currently in the market under the trade name HUMIRA and it has been approved in Europe and the United States on 2003 and 2002, respectively.

1.1.1. ADALIMUMAB Characterization

Pharmacotherapeutic group: Immunosuppressants

ATC code: L04AB04 [57]

L — ANTINEOPLASTIC AND IMMUNOMODULATING AGENTS
L04 — IMMUNOSUPPRESSANTS
L04A — IMMUNOSUPPRESSANTS
L04AB — Tumour necrosis factor alpha inhibitors

Adalimumab (HUMIRA) is a recombinant human IgG1 monoclonal antibody specific for human tumour necrosis factor (TNF) [63].

Adalimumab was created using phage display technology resulting in an antibody with human derived heavy and light chain variable regions and human IgG1 constant regions.

Adalimumab is produced by recombinant DNA technology in a mammalian cell expression system and is purified by a process that includes specific viral inactivation and removal steps [63]. It consists of 1330 amino acids and has a molecular weight of approximately 148 kilodaltons [63, 71] .

1.1.2. ADALIMUMAB Mechanism of Action

Adalimumab binds specifically to soluble TNF-alpha and blocks its interaction with p55 and p75, the cell surface TNF receptors. Adalimumab also lyses surface TNF expressing cells in vitro in the presence of complement. Adalimumab does not bind or inactivate lymphotoxin (TNF-beta) [44, 63].

Adalimumab also modulates biological responses that are induced or regulated by TNF, including changes in the levels of adhesion molecules responsible for leukocyte migration (ELAM-1, VCAM-1, and ICAM-1 with an IC50 of 1-2 X 10-10M) [63].

1.1.3. ADALIMUMAB Treatment

Table 7 – Adalimumab Treatment: Method of administration and Indications

ADALIMUMAB | *Administration:* subcutaneous injection					
U.S.	**EU**	**Indications**	**Population**	**Indications Details**	**References**
X	X	***Rheumatoid Arthritis (RA)***	Adults	– in combination with methotrexate, or on its own for moderate to severe active RA without adequate response to disease-modifying antirheumatic drugs (DMARDs) – in combination with methotrexate, or on its own for severe active rheumatoid arthritis which is getting worse and has not been treated with MTX before	[44, 63, 71, 72]
X	X	***Polyarticular Juvenile Idiopathic Arthritis***	2 – 17 years	– in combination with methotrexate, or on its own for polyarticular juvenile idiopathic arthritis which has not responded adequately to disease-modifying antirheumatic drugs (DMARDs)	[44, 63, 72]
	X	***Axial spondyloarthritis***	Adults	– severe axial spondyloarthritis without evidence in the X-ray of ankylosing spondylitis but with objective signs of inflammation, by elevated C-reactive protein (CRP) and /or magnetic resonance imaging (MRI), which has not responded adequately or are intolerant to non-steroidal anti-inflammatory drugs (NSAIDs)	[44, 63, 72]
X	X	***Ankylosing Spondylitis***	Adults	– severe active ankylosing spondylitis who have not responded adequately to other treatments;	[44, 63, 72]
X	X	***Psoriatic Arthritis***	Adults	– active and progressive psoriatic arthritis who have not responded adequately to DMARDs	[44, 63, 72]
X	X	***Psoriasis***	Adults	– severe chronic plaque psoriasis who failed to respond to or who have a contraindication to, or are intolerant to other systemic therapy including cyclosporine, methotrexate or psoralen ultraviolet-A (PUVA)	[44, 63, 72]
X	X	***Crohn's Disease***	2 – 17 years & Adults	– severe active Crohn's disease who have not responded adequately or are intolerant to conventional therapy including primary nutrition therapy (children), corticosteroids, and/or immunomodulator	[44, 63, 72]
X	X	***Ulcerative Colitis***	Adults	– moderately to severely active ulcerative colitis who have not responded adequately or are intolerant to conventional therapy including corticosteroids and 6-mercaptopurine (6-MP) or azathioprine (AZA)	[44, 63, 72]

1.1.1. ADALIMUMAB Adverse Events (as per EU Initial MA documents)

Table 8 – Adalimumab Adverse Events as per European Initial Marketing-authorisation Documents

MedDRA 17.1 System Organ Class (SOC)	AEs Detailed	References
Blood and lymphatic system disorders	– Agranulocytosis – Leukopenia – Neutropenia – Pancytopenia – Thrombocytopenia	[73]
Cardiac disorders	– Congestive heart failure (CHF) – Based on previous experience with other TNF-antagonists, Adalimumab is contraindicated in moderate to severe heart failure (NYHA class III/IV) and should be used with caution in patients with mild heart failure. – Tachycardia	[73]
Gastrointestinal disorders	– Abdominal pain	[73]
General disorders and administration site conditions	– Fatigue – Oedema – Pain – Swelling	[73]
Immune system disorders	– Anaphylactoid-type reactions	[73]
Infections and Infestations	– Bronchitis – Sepsis – Tuberculosis – Upper respiratory tract infection – Urinary tract infections	[73]
Investigations	– Decreased haemoglobin – Increased alt – increased bun – Increased coagulation time	[73]
Metabolism and nutrition disorders	– Hypercholesterolaemia – Hyperlipaemia	[73]
Musculoskeletal and connective tissue disorders	– Lupus-like syndrome	[73]
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	– Non-Hodgkin's lymphoma (NHL)	[73]
Nervous system disorders	– Demyelination – Dizziness – Headache	[73]
Renal and urinary disorders	– Haematuria	[73]
Respiratory, thoracic and mediastinal disorders	– Dyspnoea	[73]

MedDRA 17.1 **System Organ Class (SOC)**	**AEs Detailed**	**References**
Skin and subcutaneous tissue disorders	– Cutaneous abdomen rash – Erythema – Fixed drug eruption rash – Itching – Rash – Urticaria	[73]
Vascular disorders	– Haemorrhage – Hypertension	[73]

1.1.1. ADALIMUMAB Adverse Events Charts (following MA)

- ***European Data (EU database of suspected adverse drug reactions reports)***

The data here displayed was launched by EMA in the European database of suspected adverse drug reactions reports. Reports included in this database correspond to the data submitted electronically to EudraVigilance by national medicines regulatory authorities and by pharmaceutical companies that hold marketing authorisations (licences) for the medicines.

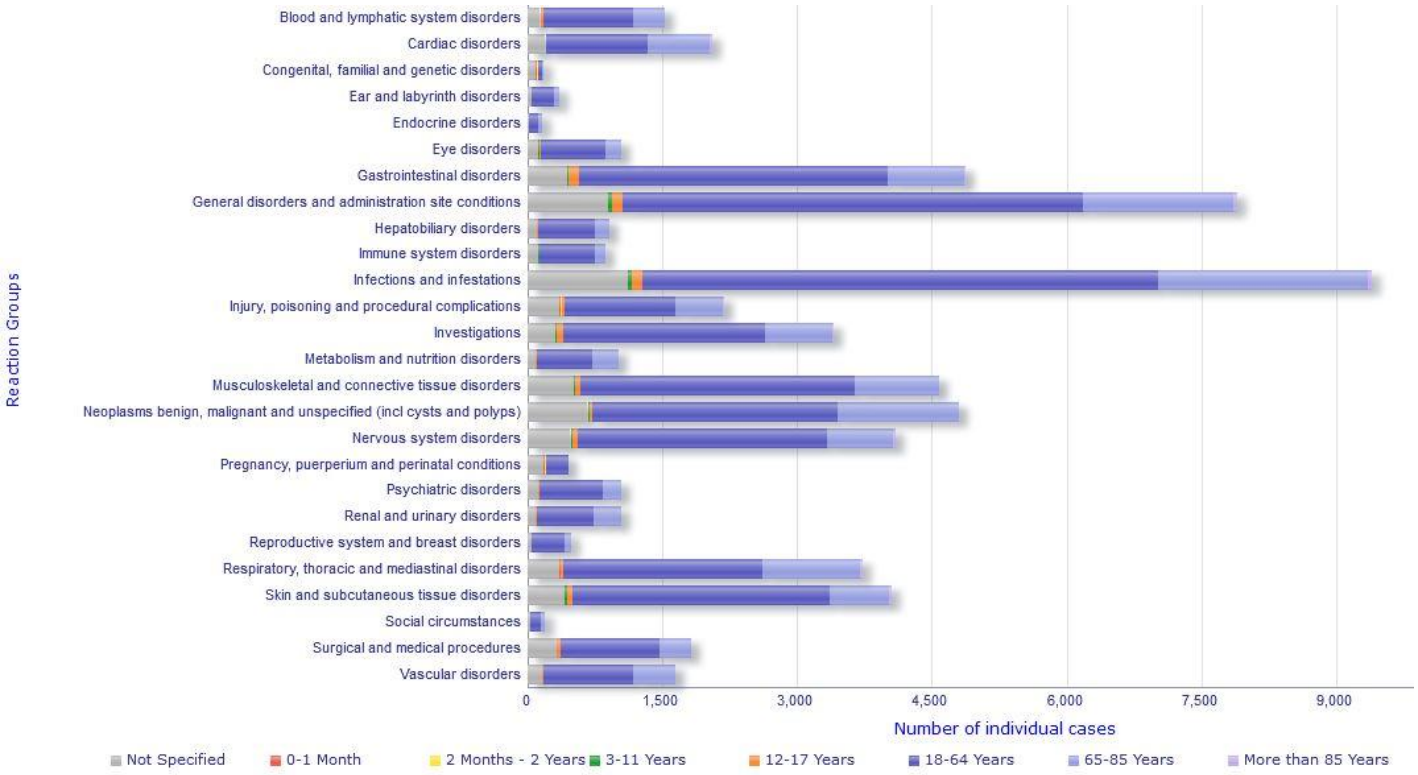

Chart 1 – Individual cases sorted by reactions groups, submitted for ADLIMUMAB (up to Oct'14)
***in* European database of suspected adverse drug reaction reports (www.adrreports.eu)**
(data extracted on 26-Nov-2014)

The chart above displays the number of cases reported for each reaction group, divided by age. The original chart (online format) enabled to pass the mouse through each bar and reveal the number of cases for a specific age. Below are the values of the biggest bars.

EU Top 10 of Individual Cases (by reactions groups):

1. Infections and Infestations (9399 cases)
2. General disordes and administration site conditions (7894 cases)
3. Gastrointestinal disorders (4880 cases)
4. Neoplasms benign, malignant and unspecified (including cysts and polyps) (4808 cases)
5. Musculoskeletal and connective tissue disorders (4588 cases)
6. Nervous system disorders (4092 cases)
7. Skin and subcutaneous tissue disorders (4049 cases)
8. Respiratory, thoracic and mediastinal disorders (3725 cases)
9. Investigations (e.g. Chest X-ray abnormal; Electocardiogram QT prolonged) (3414 cases)
10. Injury, poisoning and procedural complications (2185 cases)

- ***FDA Adverse Event Reporting System (FAERS) Data (DrugCite.com)***

The data here displayed was made available by DrugCite database. Results included in this database correspond to the collection and analysis of reported adverse performed by FDA Adverse Events Reporting System (FAERS). These reports are submitted by physicians, healthcare consumers, lawyers amongst others.

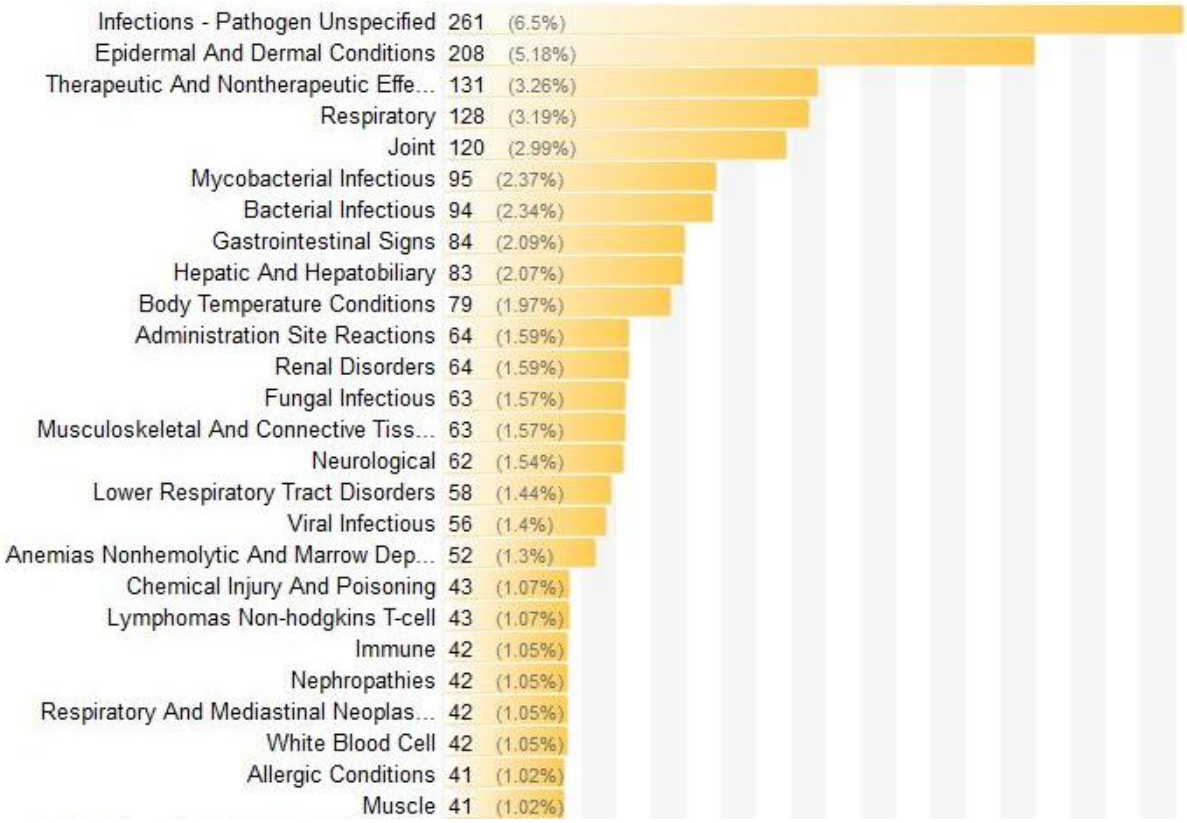

Chart 2 – Individual cases sorted by reactions groups, submitted for ADALIMUMAB and other drugs with same active substance (Q1 2004 – Q3 2012) *in* DrugCite.com (www.drugcite.com)

(data extracted on 26-Nov-2014)

The chart above displays, for each reaction group, the number of cases reported where the drug was flagged as a suspect drug causing the adverse event. All percentages (%) are the cases reported for a specific reaction group within the sum up of all reported cases in all reactions groups (percent does not represent rate related to drug utilization).

U.S. Top 10 of Individual Cases (by reactions groups):

1. Infections – Pathogen Unspecified (261 cases)
2. Epidermal And Dermal Conditions (208 cases)
3. Therapeutic And Nontherapeutic Effects (e.g. drug ineffective; intorelance) (131 cases)
4. Respiratory (128 cases)
5. Joint (e.g. Arthralgia; Joint Stiffness) (120 cases)
6. Mycobacterial Infections (95 cases)
7. Bacterial Infections (94 cases)
8. Gastrointestinal Signs (84 cases)
9. Hepatic and Hepatobiliary (83 cases)
10. Body Temperature Conditions (79 cases)

- ***Comparison of Adverse Events reported in EU and in the U.S.***

Following the analysis of the Adverse Events previously presented, it became clear that there were some differences between EU data and U.S. data. In order to have a visually enhanced perception of such differences, it was decided to create a cumulative chart which would aggregate all the data previously presented:

- Post Marketing EU data (Blue bars in the cumulative chart)
- Post Marketing U.S. data (Orange bars in the cumulative chart)
- EU data as per Initial documents for MA (MedDRA SOCs highlighted in yellow in the cumulative chart)

For aggregation purposes, a common classification had to be used and so MedDRA SOC was the selected classification for the cumulative chart below presented.

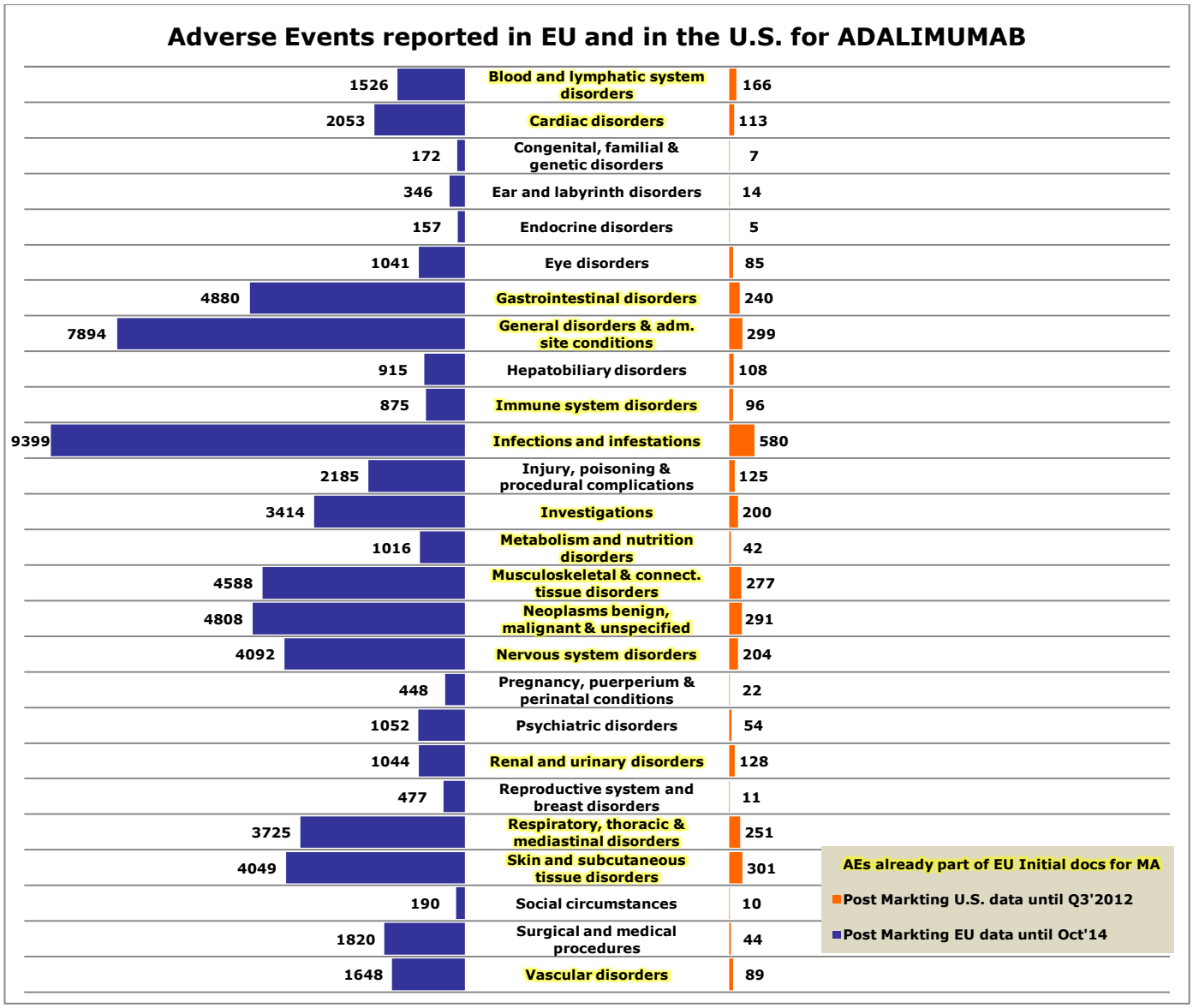

Chart 3 – Individual cases sorted by reactions groups (MedDRA 17.1 SOC), submitted for ADALIMUMAB prior and post MA in Europe and post MA the United States

Comments to ADALIMUMAB cumulative chart:

1. SOCs included in EU pre-MA reports vs SOCs included in EU post-MA reports

Main discrepancies: There are cases of SOCs with a considerable number of AE reports (>1000) in post-MA and which were not foreseen by pre-MA clinical trials data. These include: *Eye Disorders*; *Psychiatric Disorders*; *Injury, poisoning and procedural complications* & *Surgical and Medical Procedures*.

2. SOCs included in EU post-MA reports vs. SOCs included in U.S. post-MA reports

Although Adalimumab is approved in EU and in the U.S. almost for the same indications, there are some significant discrepancies in the numbers above observed.

Main discrepancies: Having EU data as reference, there were some U.S. bars which were expected to be bigger but are not. These include: *Cardiac disorders; General disorders and administration site conditions; Neoplasms benign, malignant and unspecified (including cysts and polyps)* & *Psychiatric disorders*.

Another interesting fact observed in the previous chart is that although Adalimumab is in the market of EU and U.S. for a similar time, the amount of reports seen in the U.S. data is substantially lower than those in EU data.

1.2. INFLIXIMAB

The active substance INFLIXIMAB was firstly introduced as REMICADE (trade name). Marketing Authorisation was granted on 13th Aug 1999 and alongside with REMICADE becoming a blockbuster in the market, great investment was done in order to pursue biosimilar forms of that biologic drug.

On 10th Sep 2013 Marketing Authorisation was finally granted to 2 biosimilars with the same active substance of REMICADE (Infliximab) – REMSIMA and INFLECTRA.

INFLIXIMAB's Biosimilars (REMSIMA and INFLECTRA) are considered out of the scope of the data to be here reviewed since they are Biosimilars rather than reference medicines and their approval was granted after the timeframe defined for data review: 15 years of research – 1996-2011 (please refer to 1st Objective, Task II).

1.2.1. INFLIXIMAB Characterization

Pharmacotherapeutic group: Immunosuppressants
ATC code: L04AB02 [57]

L — ANTINEOPLASTIC AND IMMUNOMODULATING AGENTS
L04 — IMMUNOSUPPRESSANTS
L04A — IMMUNOSUPPRESSANTS
L04AB — Tumour necrosis factor alpha inhibitors

Infliximab (REMICADE) is a chimeric human-murine IgG1 monoclonal antibody specific for human tumour necrosis factor alpha (TNFα) with an approximate molecular weight of 149,100 Daltons [44, 74-76].

Infliximab is produced in murine hybridoma cells by recombinant DNA technology and it is composed of human constant and murine variable regions [74, 75, 77]. INFLIXIMAB contains approximately 30% murine variable region amino acid sequence, which confers antigen-binding specificity to human TNFα. The remaining 70% correspond to a human IgG1 heavy chain constant region and a human kappa light chain constant region [77].

Infliximab is supplied as a sterile, white, lyophilized powder for intravenous infusion. Each single-use vial contains 100 mg Infliximab, 500 mg sucrose, 0.5 mg polysorbate 80, 2.2 mg monobasic sodium phosphate, monohydrate, and 6.1 mg dibasic sodium phosphate, dihydrate. Following reconstitution with 10 mL of Sterile Water for Injection, USP, the resulting pH is approximately 7.2 [74, 77].

1.2.2. INFLIXIMAB Mechanism of Action

Infliximab neutralizes the biological activity of TNFα by binding with high affinity to the soluble and transmembrane forms of TNFα and inhibits binding of TNFα with its receptors. The inhibition of TNFα biological function by the ability of Infliximab to block the interaction of TNFα with its cellular receptors, suggests that Infliximab can inhibit TNF-mediated signalling through either receptor in vivo [69].

When Infliximab is added to preformed TNF/TNF-R p55 or TNF/TNF-R75 complexes, a rapid (within 5 minutes) dissociation of TNFα from receptor is observed, with binding of dissociated TNFα to Infliximab preventing re-association with receptor [69].

Infliximab does not neutralize TNFβ (lymphotoxin α), a related cytokine that utilizes the same receptors as TNFα [74, 76].

Infliximab clears pro-inflammatory TNF from the circulation and possibly deletes aberrantly activated mTNF expressing T cells. This mechanism of action is supported by several pre-clinical experiments [76].

1.2.3. INFLIXIMAB Treatment

Table 9 – Infliximab Treatment: Method of administration and Indications

INFLIXIMAB | *Administration:* intravenous infusion					
U.S.	**EU**	**Indications**	**Population**	**Indications Details**	**References**
X	X	***Rheumatoid Arthritis (RA)***	Adults	– in combination with methotrexate for active RA without adequate response to disease-modifying antirheumatic drugs (DMARDs) – in combination with methotrexate for severe, active and progressive RA which is has not been treated with MTX before	[74, 75]
X	X	***Ankylosing Spondylitis***	Adults	– severe active ankylosing spondylitis who have not responded adequately to other treatments;	[74, 75]
X	X	***Psoriatic Arthritis***	Adults	– in combination with methotrexate, or on its own for active and progressive psoriatic arthritis who have not responded adequately to DMARDs	[74, 75]
X	X	***Psoriasis***	Adults	– severe plaque psoriasis who failed to respond to or who have a contraindication to, or are intolerant to other systemic therapy including cyclosporine, methotrexate or psoralen ultraviolet-A (PUVA)	[74, 75]
X	X	***Crohn's Disease***	2 – 17 years & Adults	– moderately to severely active Crohn's disease which has not responded adequately or is intolerant to conventional therapy including primary nutrition therapy (children), corticosteroids, and/or immunomodulator – fistulising, active Crohn's disease which has not responded adequately to conventional treatment (including antibiotics, drainage and immunosuppressive therapy)	[74, 75]
X	X	***Ulcerative Colitis***	2 – 17 years & Adults	– moderately to severely active ulcerative colitis who have not responded adequately or are intolerant to conventional therapy including corticosteroids and 6-mercaptopurine (6-MP) or azathioprine (AZA)	[74, 75]

1.1.1. INFLIXIMAB Adverse Events (as per EU Initial MA documents)

Table 10 – Infliximab Adverse Events as per European Initial Marketing-authorisation Documents

MedDRA 17.1 System Organ Class (SOC)	AEs Detailed	References
Blood and lymphatic system disorders	– Anaemia – Pancytopenia – Thrombocytopenia	[77]
Cardiac disorders	– Arrhythmia – Congestive heart failure (CHF) – Infliximab is contraindicated in patients with moderate or severe heart failure (NYHA class III/IV). Infliximab should be used with caution in patients with mild heart failure (NYHA class I/II) – Myocardial ischaemia – Pericardial effusion	[77]
Gastrointestinal disorders	– Intestinal perforation – Nausea – Pancreatitis – Stenosis	[77]
General disorders and administration site conditions	– Chest pain – Chills – Deaths – infections are the most common cause of death. Sepsis and localised infections (like pneumonia) were also cause of some deaths – Fever	[77]
Hepatobiliary disorders	– Abnormal hepatic function – Cholecystitis – Hepatitis – Hepatocellular damage	[77]
Immune system disorders	– Delayed Hypersensitivity (including Serum Sickness-like) Reactions	[77]
Infections and Infestations	– Aspergillosis, listeriosis – Cellulitis – Coccidioides – Cryptococcus infection – Cytomegalovirus (CMV) – Histoplasmosis – Pneumocystis carinii pneumonia – Pneumonia – Pyelonephritis – Sepsis – Sinusitis – Systemic candidiasis – Tuberculosis – Upper respiratory tract infection	[77]

MedDRA 17.1 **System Organ Class (SOC)**	**AEs Detailed**	**References**
Investigations	– Antibodies to Infliximab (various data show an association between antibodies to Infliximab and diminished degree of efficacy and increased incidence of infusion reactions) – Antinuclear antibodies (ANA) increased – Approximately half of Infliximab-treated patients in clinical studies who were ANA negative at baseline developed a positive ANA during the study. There were few cases that newly tested positive for anti-dsDNA antibodies. – Changes in white blood cells count	[77]
Musculoskeletal and connective tissue disorders	– Lupus-like syndrome – mostly for patients who became positive for anti-dsDNA	[77]
Nervous system disorders	– Demyelination – suggestive of multiple sclerosis or localized demyelination conditions such as optic neuritis – Headache	[77]
Respiratory, thoracic and mediastinal disorders	– Cough – Dyspnoea – Interstitial pneumonitis/ fibrosis	[77]
Skin and subcutaneous tissue disorders	– Pruritus – Rash – Urticaria	[77]
Vascular disorders	– Hyper/hypotension – Vasculitis	[77]

1.1.1. INFLIXIMAB Adverse Events Charts (following MA)

- ***European Data (EU database of suspected adverse drug reactions reports)***

The data here displayed was launched by EMA in the European database of suspected adverse drug reactions reports. Reports included in this database correspond to the data submitted electronically to EudraVigilance by national medicines regulatory authorities and by pharmaceutical companies that hold marketing authorisations (licences) for the medicines.

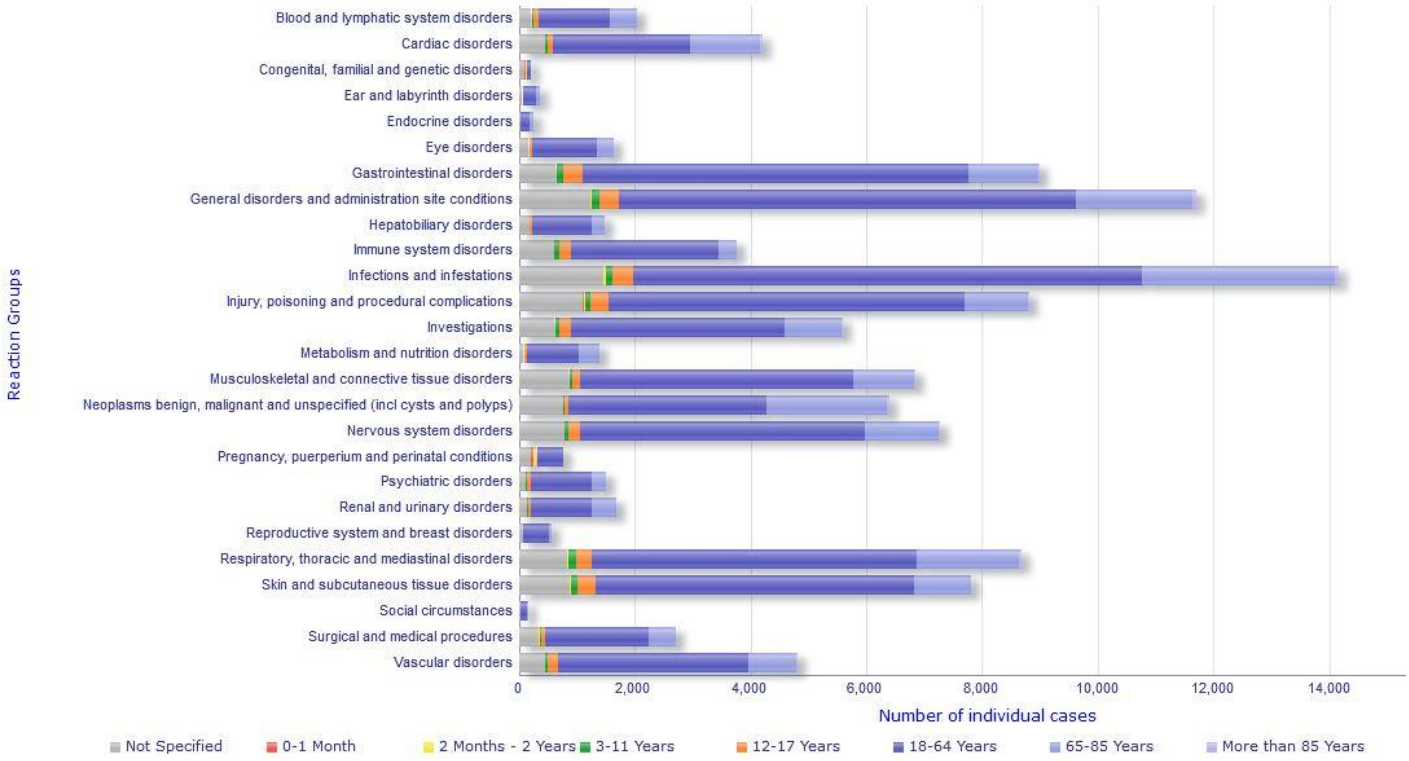

Chart 4 – Individual cases sorted by reactions groups, submitted for INFLIXIMAB (up to Oct'14)
***in* European database of suspected adverse drug reaction reports (www.adrreports.eu)**
(data extracted on 26-Nov-2014)

The chart above displays the number of cases reported for each reaction group, divided by age. The original chart (online format) enabled to pass the mouse through each bar and reveal the number of cases for a specific age. Below are the values of the biggest bars.

EU Top 10 of Individual Cases (by reactions groups):

1. Infections and Infestations (14171 cases)
2. General disordes and administration site conditions (11700 cases)
3. Gastrointestinal disorders (8995 cases)
4. Injury, poisoning and procedural complications (8826 cases)
5. Respiratory, thoracic and mediastinal disorders (8673 cases)
6. Skin and subcutaneous tissue disorders (7821 cases)
7. Nervous system disorders (7284 cases)
8. Musculoskeletal and connective tissue disorders (6840 cases)
9. Neoplasms benign, malignant and unspecified (including cysts and polyps) (6395 cases)
10. Investigations (e.g. Chest X-ray abnormal; Electocardiogram QT prolonged) (5597 cases)

- ***FDA Adverse Event Reporting System (FAERS) Data (DrugCite.com)***

The data here displayed was made available by DrugCite database. Results included in this database correspond to the collection and analysis of reported adverse performed by FDA Adverse Events Reporting System (FAERS). These reports are submitted by physicians, healthcare consumers, lawyers amongst others.

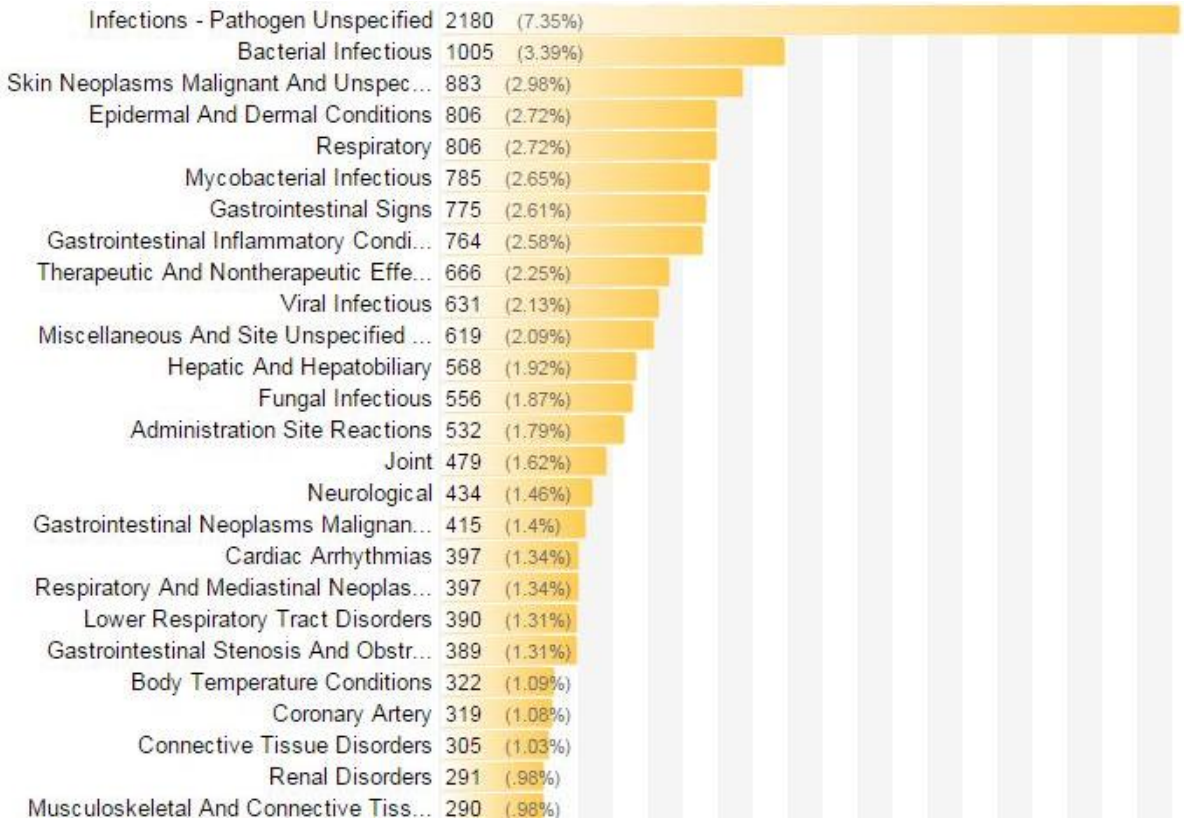

Chart 5 – Individual cases sorted by reactions groups, submitted for INFLIXIMAB and other drugs with same active substance (Q1 2004 – Q3 2012) *in* DrugCite.com (www.drugcite.com)

(data extracted on 26-Nov-2014)

The chart above displays, for each reaction group, the number of cases reported where the drug was flagged as a suspect drug causing the adverse event. All percentages (%) are the cases reported for a specific reaction group within the sum up of all reported cases in all reactions groups (percent does not represent rate related to drug utilization).

U.S. Top 10 of Individual Cases (by reactions groups):

1. Infections – Pathogen Unspecified (2180 cases)
2. Bacterial Infectious (1005 cases)
3. Skin Neoplasms Malignant and Unspecified (883 cases)
4. Epidermal and Dermal Conditions (806 cases)
5. Respiratory (806 cases)
6. Mycobacterial Infectious (785 cases)
7. Gastrointestinal Signs (775 cases)
8. Gastrointestinal Inflammatory Conditions (764 cases)
9. Therapeutic and Nontherapeutic Effects (666 cases)
10. Viral Infectious (631 cases)

- ***Comparison of Adverse Events reported in EU and in the U.S.***

Following the analysis of the Adverse Events previously presented, it became clear that there were some differences between EU data and U.S. data. In order to have a visually enhanced perception of such differences, it was decided to create a cumulative chart which would aggregate all the data previously presented:

- Post Marketing EU data (Blue bars in the cumulative chart)
- Post Marketing U.S. data (Orange bars in the cumulative chart)
- EU data as per Initial documents for MA (MedDRA SOCs highlighted in yellow in the cumulative chart)

For aggregation purposes, a common classification had to be used and so MedDRA SOC was the selected classification for the cumulative chart below presented.

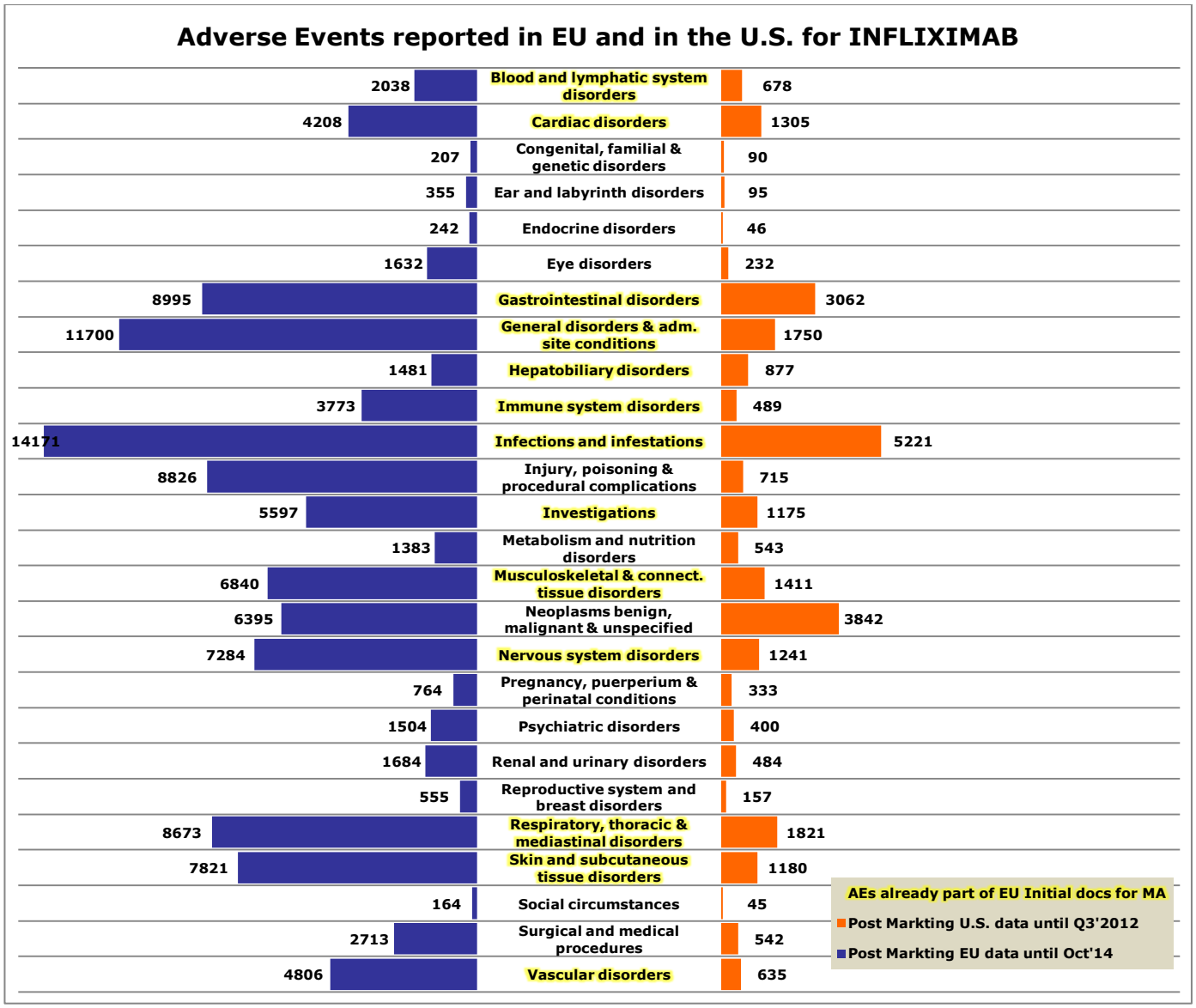

Chart 6 – Individual cases sorted by reactions groups (MedDRA 17.1 SOC), submitted for INFLIXIMAB prior and post MA in Europe and post MA the United States

Comments to INFLIXIMAB cumulative chart:

1. SOCs included in EU pre-MA reports vs SOCs included in EU post-MA reports

Main discrepancies: There are cases of SOCs with a considerable number of AE reports (>2000) in post-MA and which were not foreseen by pre-MA clinical trials data. These include*: Injury, poisoning and procedural complications* & *Surgical and Medical Procedures*.

2. SOCs included in EU post-MA reports vs. SOCs included in U.S. post-MA reports

Although Infliximab is approved in EU and in the U.S. for the same indications, there are some significant discrepancies in the numbers above observed.

Main discrepancies: Having EU data as reference, there were some U.S. bars which were expected to be bigger but are not. These include: *General disorders and administration site conditions*; *Immune System Disorders*; *Injury, poisoning and procedural complications*; *Musculoskeletal and connective tissue disorders*; *Nervous system disorders* & *Vascular disorders*.

1.2. GOLIMUMAB

1.2.1. GOLIMUMAB Characterization

Pharmacotherapeutic group: Immunosuppressants
ATC code: L04AB06 [57]

L — ANTINEOPLASTIC AND IMMUNOMODULATING AGENTS
L04 — IMMUNOSUPPRESSANTS
L04A — IMMUNOSUPPRESSANTS
L04AB — Tumour necrosis factor alpha inhibitors

Golimumab (trade name SIMPONI) is a human IgG1 k, monoclonal antibody specific for human tumour necrosis factor alpha (TNF-a) that exhibits multiple glycoforms with molecular masses of approximately 150 to 151 kilodaltons [78].

Golimumab was created using genetically engineered mice immunized with human TNF, resulting in an antibody with human-derived antibody variable and constant regions. Golimumab is produced by recombinant cell line cultured by continuous perfusion and is purified by a series of steps that includes measures to inactivate and remove viruses [78].

1.2.2. GOLIMUMAB Mechanism of Action

Golimumab is a human IgG1κ monoclonal antibody that binds to both the soluble and transmembrane bioactive forms of human TNFα. This interaction prevents the binding of TNFα to its receptors, thereby inhibiting the biological activity of TNFα (a cytokine protein). There was no evidence of the Golimumab antibody binding to other TNF superfamily ligands; in particular, the Golimumab antibody did not bind or neutralize human lymphotoxin. Golimumab did not lyse human monocytes expressing transmembrane TNF in the presence of complement or effector cells.

Elevated TNFα levels in the blood, synovium, and joints have been implicated in the pathophysiology of several chronic inflammatory diseases such as rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. TNFα is an important mediator of the articular inflammation that is characteristic of these diseases. Golimumab modulated the in vitro biological effects mediated by TNF in several bioassays, including the expression of adhesion proteins responsible for leukocyte infiltration (E-selectin, ICAM-1 and VCAM-1) and the secretion of pro-inflammatory cytokines (IL-6, IL-8, O-CSF and GM-CSF) [78].

1.2.3. GOLIMUMAB Treatment

Table 11 – Golimumab Treatment: Method of administration and Indications

GOLIMUMAB | *Administration:* subcutaneous injection					
U.S.	**EU**	**Indications**	**Population**	**Indications Details**	**References**
X	X	***Rheumatoid Arthritis (RA)***	Adults	– in combination with methotrexate for moderate to severe, active RA without adequate response to disease-modifying antirheumatic drugs (DMARDs) – in combination with methotrexate for severe, active and progressive RA which is has not been treated with MTX before	[78, 79]
X	X	***Ankylosing Spondylitis***	Adults	– severe, active ankylosing spondylitis which has not responded adequately to conventional therapy;	[78, 79]
X	X	***Psoriatic Arthritis***	Adults	– in combination with methotrexate, or on its own for active and progressive psoriatic arthritis which has not responded adequately to DMARDs	[78, 79]
X	X	***Ulcerative Colitis***	Adults	– moderately to severely active ulcerative colitis who have not responded adequately or are intolerant to conventional therapy including corticosteroids and 6-mercaptopurine (6-MP) or azathioprine (AZA)	[78, 79]

1.1.1. GOLIMUMAB Adverse Events (as per EU Initial MA documents)

Table 12 – Golimumab Adverse Events as per European Initial Marketing-authorisation Documents

MedDRA 17.1 **System Organ Class (SOC)**	**AEs Detailed**	**References**
Cardiac disorders	– Arrhythmia – Congestive heart failure (CHF) – it was decided the contraindication for patients with moderate to severe heart failure (NYHA class iii/iv) and a special warning for use of Golimumab in CHF – Ischemic coronary artery disorders	[80]
General disorders and administration site conditions	– Deaths – among these deaths, there were infections, malignancy, cardiac and hepatic events which were possibly related to Golimumab treatment.	[80]
Hepatobiliary disorders	– Acute cholecystitis – Hepatomegaly – Toxic hepatitis	[80]
Immune system disorders	– Hypersensitivity – Rare cases of serum sickness	[80]

MedDRA 17.1 **System Organ Class (SOC)**	**AEs Detailed**	**References**
Infections and Infestations	– Abscess – Cellulitis – Infectious mononucleosis – Otitis media chronic – Pneumonia – Sepsis (sepsis and urosepsis) – Tuberculosis – Upper respiratory tract infection	[80]
Injury, poisoning and procedural complications	– Injection reactions	[80]
Investigations	– Antinuclear antibodies (ANA) increased – the proportion of subjects who were negative for antinuclear antibodies (ANA) at baseline, but who had a newly positive ANA test result during the studies, was increased in the majority of Golimumab groups. There were few cases that newly tested positive for anti-dsDNA antibodies. – Liver enzyme elevations – some cases of patients experiencing alt increase – Neutrophils reduced	[80]
Musculoskeletal and connective tissue disorders	– Lupus erythematosus – Lupus-like syndrome	[80]
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	– Lymphoma – Nonmelanoma skin cancers	[80]
Nervous system disorders	– Demyelination	[80]
Psychiatric disorders	– Anxiety – Depression – Insomnia – Suicide attempt/ideation	[80]
Respiratory, thoracic and mediastinal disorders	– Fibrosing alveolitis – Interstitial lung disease – Pneumonitis	[80]
Skin and subcutaneous tissue disorders	– Erythema – Pustular psoriasis – Rash – Urticaria	[80]
Vascular disorders	– Hypertension – Vasculitis	[80]

1.1.1. GOLIMUMAB Adverse Events Charts (following MA)

- ***European Data (EU database of suspected adverse drug reactions reports)***

The data here displayed was launched by EMA in the European database of suspected adverse drug reactions reports. Reports included in this database correspond to the data submitted electronically to EudraVigilance by national medicines regulatory authorities and by pharmaceutical companies that hold marketing authorisations (licences) for the medicines.

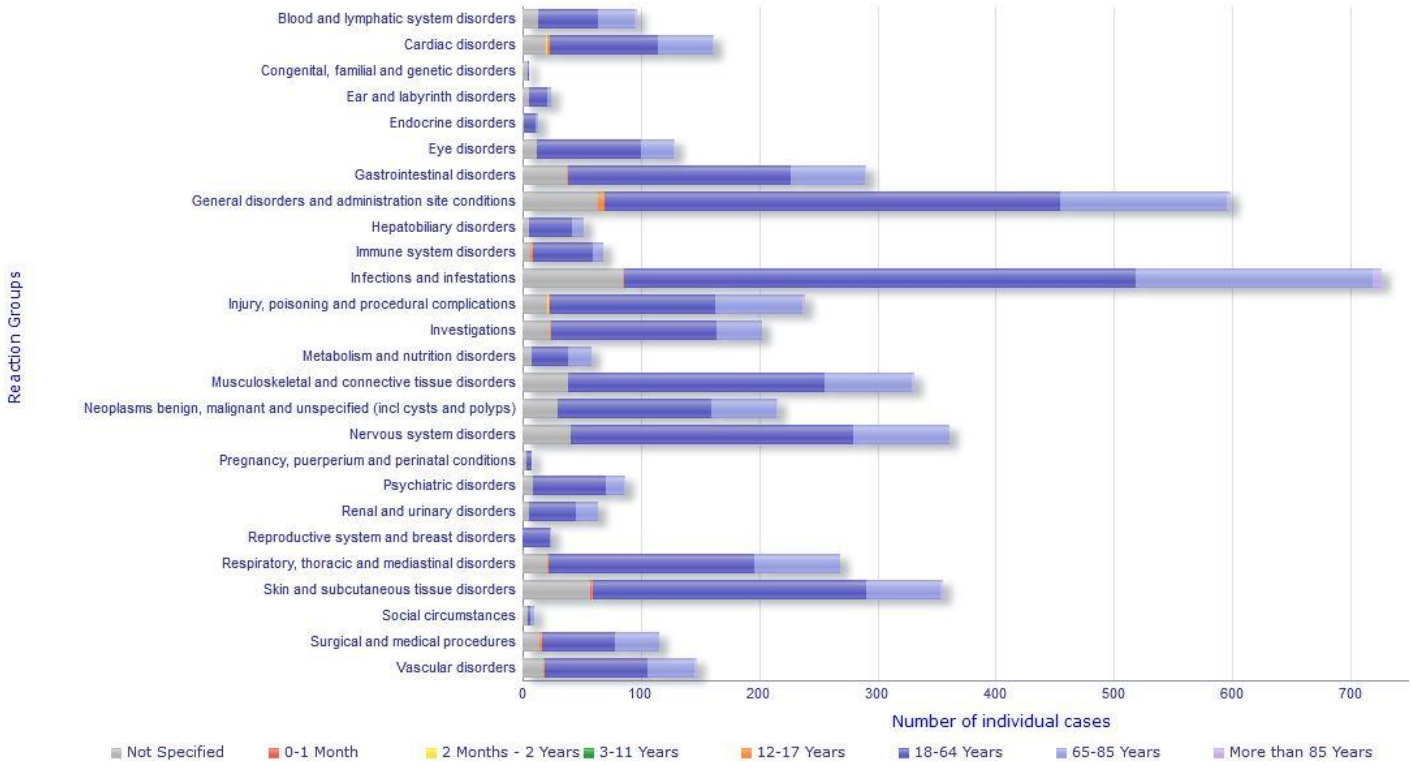

Chart 7 – Individual cases sorted by reactions groups, submitted for GOLIMUMAB (up to Oct'14) *in* European database of suspected adverse drug reaction reports (www.adrreports.eu) (data extracted on 26-Nov-2014)

The chart above displays the number of cases reported for each reaction group, divided by age. The original chart (online format) enabled to pass the mouse through each bar and reveal the number of cases for a specific age. Below are the values of the biggest bars.

EU Top 10 of Individual Cases (by reactions groups):

1. Infections and Infestations (726 cases)
2. General disordes and administration site conditions (599 cases)
3. Nervous system disorders (361 cases)
4. Skin and subcutaneous tissue disorders (355 cases)
5. Musculoskeletal and connective tissue disorders (331 cases)
6. Gastrointestinal disorders (291 cases)
7. Respiratory, thoracic and mediastinal disorders (269 cases)
8. Injury, poisoning and procedural complications (239 cases)
9. Neoplasms benign, malignant and unspecified (including cysts and polyps) (216 cases)
10. Investigations (e.g. Chest X-ray abnormal; Electocardiogram QT prolonged) (203 cases)

- ***FDA Adverse Event Reporting System (FAERS) Data (DrugCite.com)***

The data here displayed was made available by DrugCite database. Results included in this database correspond to the collection and analysis of reported adverse performed by FDA Adverse Events Reporting System (FAERS). These reports are submitted by physicians, healthcare consumers, lawyers amongst others.

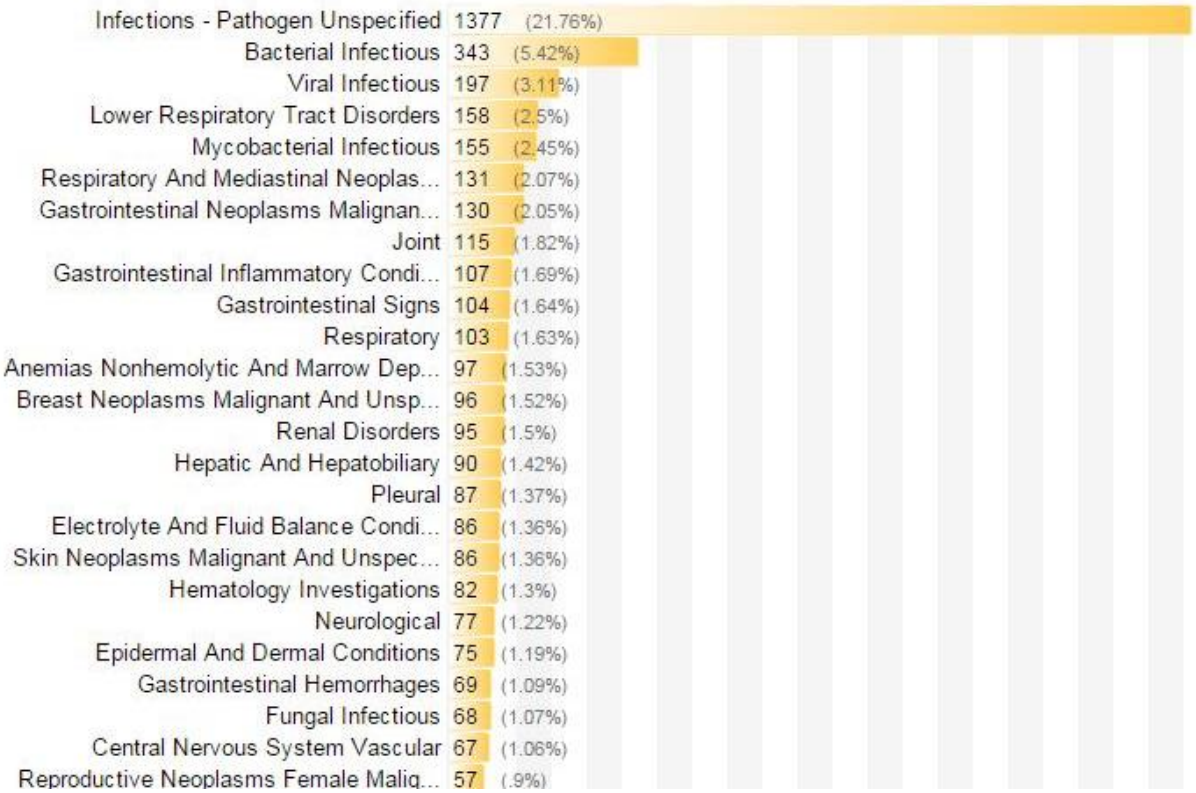

Chart 8 – Individual cases sorted by reactions groups, submitted for GOLIMUMAB and other drugs with same active substance (Q1 2004 – Q3 2012) *in* DrugCite.com (www.drugcite.com)

(data extracted on 26-Nov-2014)

The chart above displays, for each reaction group, the number of cases reported where the drug was flagged as a suspect drug causing the adverse event. All percentages (%) are the cases reported for a specific reaction group within the sum up of all reported cases in all reactions groups (percent does not represent rate related to drug utilization).

U.S. Top 10 of Individual Cases (by reactions groups):

1. Infections – Pathogen Unspecified (1377 cases)
2. Bacterial Infectious (343 cases)
3. Viral Infectious (197 cases)
4. Lower Respiratory Disorders (158 cases)
5. Mycobacterial Infectious (155 cases)
6. Respiratory and Mediastinal Neoplasms Malignant and Unspecified (131 cases)
7. Gastrointestinal Neoplasms Malignant and Unspecified (130 cases)
8. Joint (e.g. Arthralgia; Joint Stiffness) (115 cases)
9. Gastrointestinal Inflammatory Conditions (107 cases)
10. Gastrointestinal Signs (104 cases)

- ***Comparison of Adverse Events reported in EU and in the U.S.***

Following the analysis of the Adverse Events previously presented, it became clear that there were some differences between EU data and U.S. data. In order to have a visually enhanced perception of such differences, it was decided to create a cumulative chart which would aggregate all the data previously presented:

- Post Marketing EU data (Blue bars in the cumulative chart)
- Post Marketing U.S. data (Orange bars in the cumulative chart)
- EU data as per Initial documents for MA (MedDRA SOCs highlighted in yellow in the cumulative chart)

For aggregation purposes, a common classification had to be used and so MedDRA SOC was the selected classification for the cumulative chart below presented.

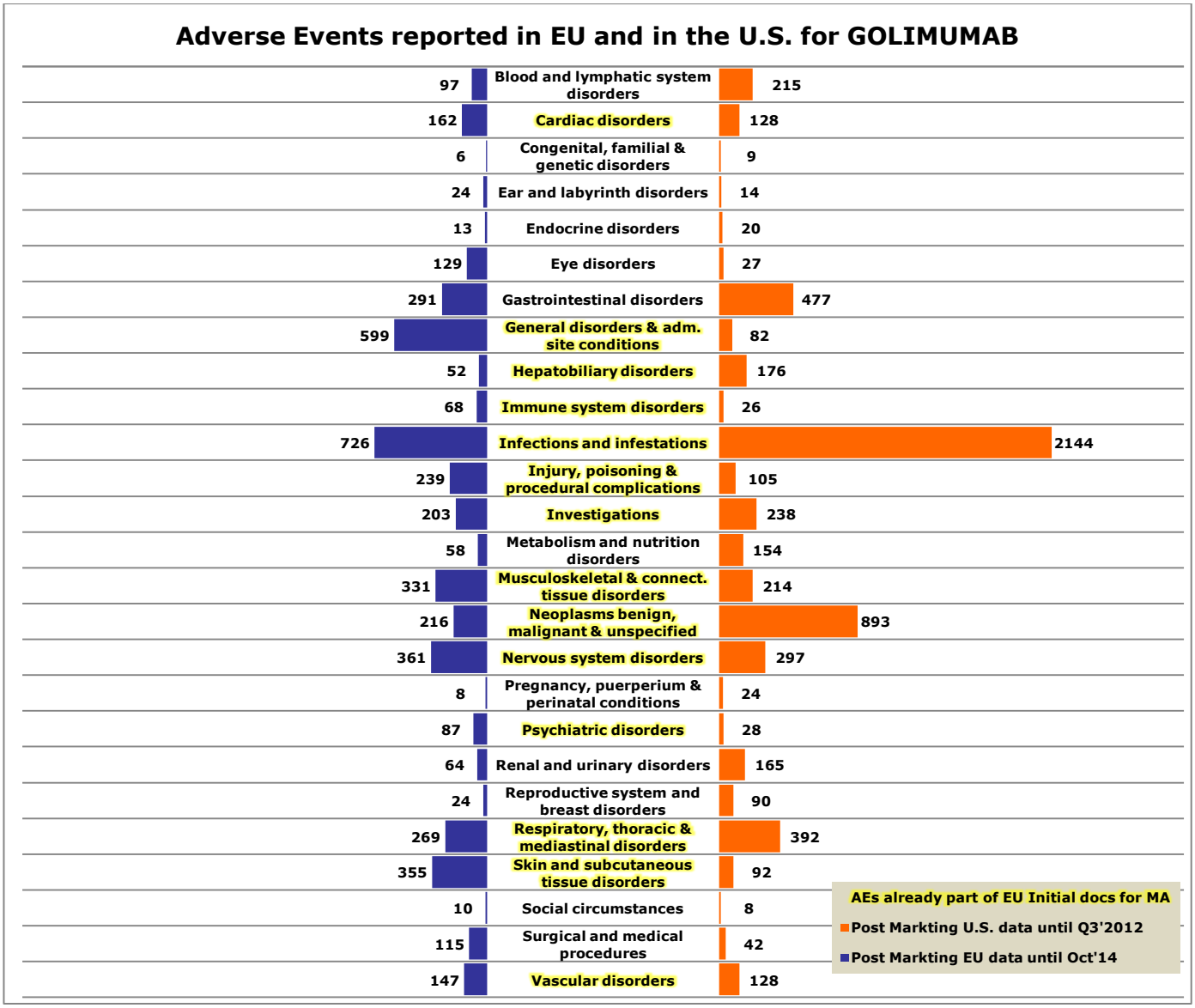

Chart 9 – Individual cases sorted by reactions groups (MedDRA 17.1 SOC), submitted for GOLIMUMAB prior and post MA in Europe and post MA the United States

Comments to GOLIMUMAB cumulative chart:

1. SOCs included in EU pre-MA reports vs SOCs included in EU post-MA reports

Main discrepancies: There are cases of SOCs with a considerable number of AE reports (>150) in post-MA and which were not foreseen by pre-MA clinical trials data. These include*: Eye disorders*; *Gastrointestinal disorders*& *Surgical and Medical Procedures*.

2. SOCs included in EU post-MA reports vs. SOCs included in U.S. post-MA reports

Although Golimumab is approved in EU and in the U.S. for the same indications, there are some significant discrepancies in the numbers above observed.

Main discrepancies: Having EU data as reference, there were some U.S. bars which were expected to be bigger but are not. These include: *Cardiac disorders*; *General disorders and administration site conditions*; *Immune system disorders*; *Injury, poisoning and procedural complications*; *Musculoskeletal and connective tissue disorders*; *Nervous system disorders*; *Psychiatric disorders* & *Skin and subcutaneous tissue disorders*.

Based on EU data, there were also some U.S. bars which were expected to be smaller: *Neoplasms benign, malignant and unspecified (including cysts and polyps)*.

1.2. CERTOLIZUMAB PEGOL

Certolizumab pegol (trade name CIMZIA) is currently in the market with different indications for Europe and for the United Sates.

Certolizumab pegol firstly applied for Market Authorisation in Europe for treatment of Crohn's Disease. This application was refused on 21st May 2008 on the grounds of safety concerns and insignificant effectiveness [81].

FDA, on another hand, issued in 22nd April 2008 the approval for Certolizumab pegol for the treatment of Crohn's Disease, based on safety and efficacy data from clinical trials in more than 1 500 patients with Crohn's disease [82].

Following that disapproval/approval, respectively, Certolizumab pegol applied for another number of indications (to be discussed further on) and it has now approvals granted both in Europe and U.S. Marketing Authorisation in Europe was granted on 1st October 2009, for the treatment of Arthritis Rheumatoid.

1.2.1. CERTOLIZUMAB PEGOL Characterization

Pharmacotherapeutic group: Immunosuppressants
ATC code: L04AB05 [57]

L — ANTINEOPLASTIC AND IMMUNOMODULATING AGENTS
L04 — IMMUNOSUPPRESSANTS
L04A — IMMUNOSUPPRESSANTS
L04AB — Tumour necrosis factor alpha inhibitors

Certolizumab pegol (CIMZIA) is a recombinant, humanised antibody Fab' fragment, specific for human tumour necrosis factor alpha (TNFα), conjugated to an approximately 40kDa polyethylene glycol (PEG2MAL40K).

The Fab' fragment is manufactured in E. coli and is then subjected to purification and conjugation to PEG2MAL40K, to generate Certolizumab pegol. The Fab' fragment is composed of a light chain with 214 amino acids and a heavy chain with 229 amino acids.

The molecular weight of Certolizumab pegol is approximately 91 kiloDaltons [83].

1.2.2. CERTOLIZUMAB PEGOL Mechanism of Action

Certolizumab pegol is a pegylated humanised Fab' fragment of an anti-TNF-alpha monoclonal antibody that binds to free and membrane-bound human TNFα with a KD of 90pM and neutralizes its activity.

TNFα is a key pro-inflammatory cytokine with a central role in inflammatory processes.

Certolizumab pegol selectively neutralizes TNFα but does not neutralize lymphotoxin α (TNFβ).

Certolizumab pegol cross-reacts poorly with TNF from rodents and rabbits, therefore in vivo efficacy was evaluated using animal models in which human TNFα was the physiologically active molecule.

Certolizumab pegol was shown to neutralize membrane-associated and soluble human TNFα in a dose-dependent manner. Incubation of monocytes with Certolizumab pegol resulted in a dose-dependent inhibition of LPS-induced TNFα and IL-1β production in human monocytes.

Certolizumab pegol does not contain a fragment crystallisable (Fc) region, which is normally present in a complete antibody, and therefore does not fix complement or cause antibody-dependent cell-mediated cytotoxicity in vitro. It does not induce apoptosis in vitro in human peripheral blood-derived monocytes or lymphocytes, nor does Certolizumab pegol induce neutrophil degranulation. Certolizumab pegol clearly shows that the efficacy of anti-TNF-alpha antibodies does not require the apoptosis for the efficacy in response and maintenance of remission.

A tissue reactivity study was carried out ex vivo to evaluate potential cross-reactivity of Certolizumab pegol with cryosections of normal human tissues. Certolizumab pegol showed no reactivity with a designated standard panel of normal human tissues [83, 84].

1.2.3. CERTOLIZUMAB PEGOL Treatment

Table 13 – Certolizumab pegol Treatment: Method of administration and Indications

CERTOLIZUMAB PEGOL | *Administration:* subcutaneous injection					
U.S.	**EU**	**Indications**	**Population**	**Indications Details**	**References**
X	X	***Rheumatoid Arthritis (RA)***	Adults	– in combination with methotrexate, or on its own for moderate to severe, active RA without adequate response to disease-modifying antirheumatic drugs (DMARDs)	[83, 85]
	X	***Axial spondyloarthritis***	Adults	– severe axial spondyloarthritis without evidence in the X-ray of ankylosing spondylitis but with objective signs of inflammation, by elevated C-reactive protein (CRP) and /or magnetic resonance imaging (MRI), which has not responded adequately or are intolerant to non-steroidal anti-inflammatory drugs (NSAIDs)	[85]
	X	***Ankylosing Spondylitis***	Adults	– severe, active ankylosing spondylitis which has not responded adequately to, or are intolerant to nonsteroidal anti-inflammatory drugs (NSAIDs)	[85]
	X	***Psoriatic Arthritis***	Adults	– in combination with methotrexate, or on its own for active and progressive psoriatic arthritis which has not responded adequately to DMARDs	[85]
X		***Crohn's Disease***	Adults	– moderately to severely active Crohn's disease which has not responded adequately to conventional therapy including corticosteroids, and/or immunomodulator	[83]

1.1.1. CERTOLIZUMAB PEGOL Adverse Events (as per EU Initial MA documents)

Table 14 – Certolizumab Adverse Events as per European Initial Marketing-authorisation Documents

MedDRA 17.1 System Organ Class (SOC)	AEs Detailed	References
Blood and lymphatic system disorders	– Anaemia – Eosinophilia	[86]
Cardiac disorders	– Congestive heart failure – the use of anti-TNF agents is contraindicated in subjects with moderate to severe heart failure	[86]
Gastrointestinal disorders	– Gastrointestinal haemorrhage – Melaena – Melaena (due to epistaxis) – Rectal haemorrhage	[86]
General disorders and administration site conditions	– Deaths – The majority of deaths were from cardiac and infectious causes, a pattern similar to that observed in other biologically-treated RA subject populations – Hepatobiliary investigations – increased alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transferase	[86]
Immune system disorders	– Hypersensitivity – Serum sickness	[86]
Infections and Infestations	– Herpes infections – Lower respiratory tract infections – Lung infections – Nasopharyngitis – Opportunistic infections – Reactivation of viral infections – Tuberculosis (including pulmonary tuberculosis, disseminated tuberculosis, peritoneal tuberculosis, lymph node tuberculosis, and tuberculosis pleurisy) – Upper respiratory tract infection – Urinary tract infections	[86]
Injury, poisoning and procedural complications	– Post-procedural haemorrhage	[86]
Investigations	– Activated partial thromboplastin time (APTT) prolonged	[86]
Musculoskeletal and connective tissue disorders	– Lupus-like events	[86]

MedDRA 17.1 **System Organ Class (SOC)**	**AEs Detailed**	**References**
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	– B cell lymphoma – Basocellular carcinoma of the skin – Colon cancer – Hepatic neoplasm – Lung cancer – Metastases to CNS – Oesophageal carcinoma – Testicular cancer – Tongue neoplasm – Uterine cancer	[86]
Renal and urinary disorders	– Haematuria	[86]
Reproductive system and breast disorders	– Menorrhagia – Uterine haemorrhage	[86]
Respiratory, thoracic and mediastinal disorders	– Bleeding nasal polyp – Lung infiltration	[86]
Skin and subcutaneous tissue disorders	– Purpura	[86]
Vascular disorders	– Haematoma – Hypertensive events	[86]

1.1.1. CERTOLIZUMAB PEGOL Adverse Events Charts (following MA)

- ***European Data (EU database of suspected adverse drug reactions reports)***

The data here displayed was launched by EMA in the European database of suspected adverse drug reactions reports. Reports included in this database correspond to the data submitted electronically to EudraVigilance by national medicines regulatory authorities and by pharmaceutical companies that hold marketing authorisations (licences) for the medicines.

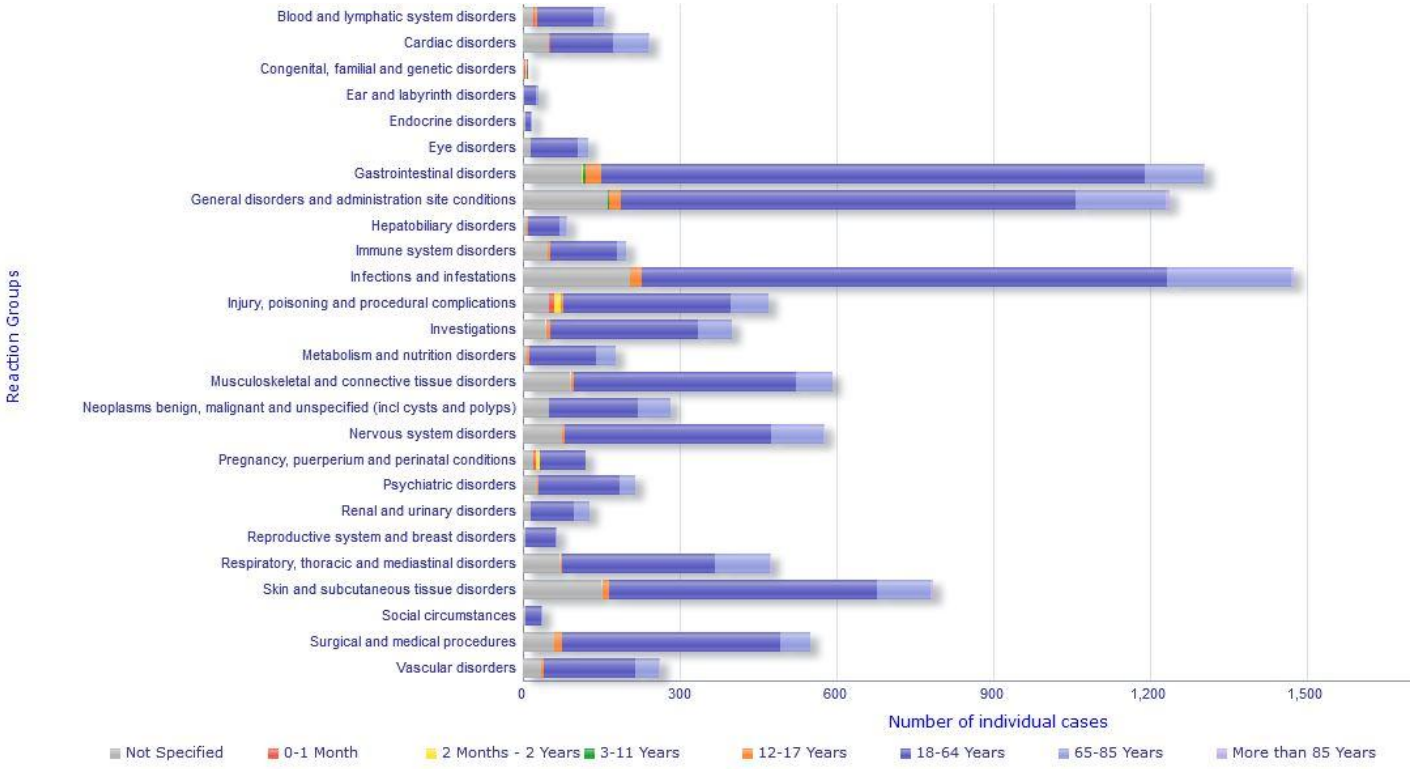

Chart 10 – Individual cases sorted by reactions groups, submitted for CERTOLIZUMAB (up to Oct'14)
***in* European database of suspected adverse drug reaction reports (www.adrreports.eu)**
(data extracted on 26-Nov-2014)

The chart above displays the number of cases reported for each reaction group, divided by age. The original chart (online format) enabled to pass the mouse through each bar and reveal the number of cases for a specific age. Below are the values of the biggest bars.

EU Top 10 of Individual Cases (by reactions groups):

1. Infections and Infestations (1475 cases)
2. Gastrointestinal disorders (1304 cases)
3. General disordes and administration site conditions (1237 cases)
4. Skin and subcutaneous tissue disorders (784 cases)
5. Musculoskeletal and connective tissue disorders (593 cases)
6. Nervous system disorders (578 cases)
7. Surgical and medical procedures (549 cases)
8. Respiratory, thoracic and mediastinal disorders (474 cases)
9. Injury, poisoning and procedural complications (470 cases)
10. Investigations (e.g. Chest X-ray abnormal; Electocardiogram QT prolonged) (400 cases)

- ***FDA Adverse Event Reporting System (FAERS) Data (DrugCite.com)***

The data here displayed was made available by DrugCite database. Results included in this database correspond to the collection and analysis of reported adverse performed by FDA Adverse Events Reporting System (FAERS). These reports are submitted by physicians, healthcare consumers, lawyers amongst others.

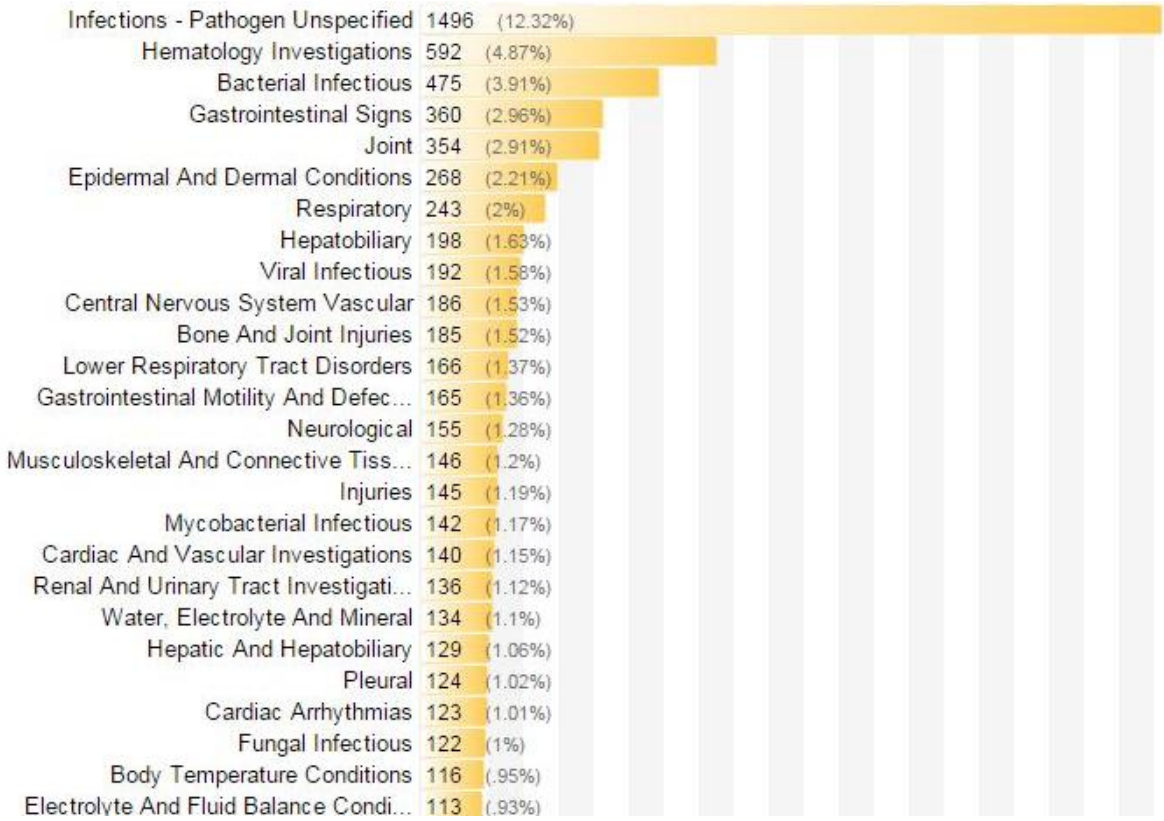

Chart 11 – Individual cases sorted by reactions groups, submitted for CERTOLIZUMAB and other drugs with same active substance (Q1 2004 – Q3 2012) *in* DrugCite.com (www.drugcite.com)

(data extracted on 26-Nov-2014)

The chart above displays, for each reaction group, the number of cases reported where the drug was flagged as a suspect drug causing the adverse event. All percentages (%) are the cases reported for a specific reaction group within the sum up of all reported cases in all reactions groups (percent does not represent rate related to drug utilization).

U.S. Top 10 of Individual Cases (by reactions groups):

1. Infections – Pathogen Unspecified (1496 cases)
2. Hematology Investigations (592 cases)
3. Bacterial Infectious (475 cases)
4. Gastrointestinal Signs (360 cases)
5. Joint (e.g. Arthralgia; Joint Stiffness) (354 cases)
6. Epidermal And Dermal Conditions (268 cases)
7. Respiratory (243 cases)
8. Hepatobiliary (198 cases)
9. Viral Infectious (192 cases)
10. Central Nervous System Vascular (186 cases)

- ***Comparison of Adverse Events reported in EU and in the U.S.***

Following the analysis of the Adverse Events previously presented, it became clear that there were some differences between EU data and U.S. data. In order to have a visually enhanced perception of such differences, it was decided to create a cumulative chart which would aggregate all the data previously presented:

- Post Marketing EU data (Blue bars in the cumulative chart)
- Post Marketing U.S. data (Orange bars in the cumulative chart)
- EU data as per Initial documents for MA (MedDRA SOCs highlighted in yellow in the cumulative chart)

For aggregation purposes, a common classification had to be used and so MedDRA SOC was the selected classification for the cumulative chart below presented.

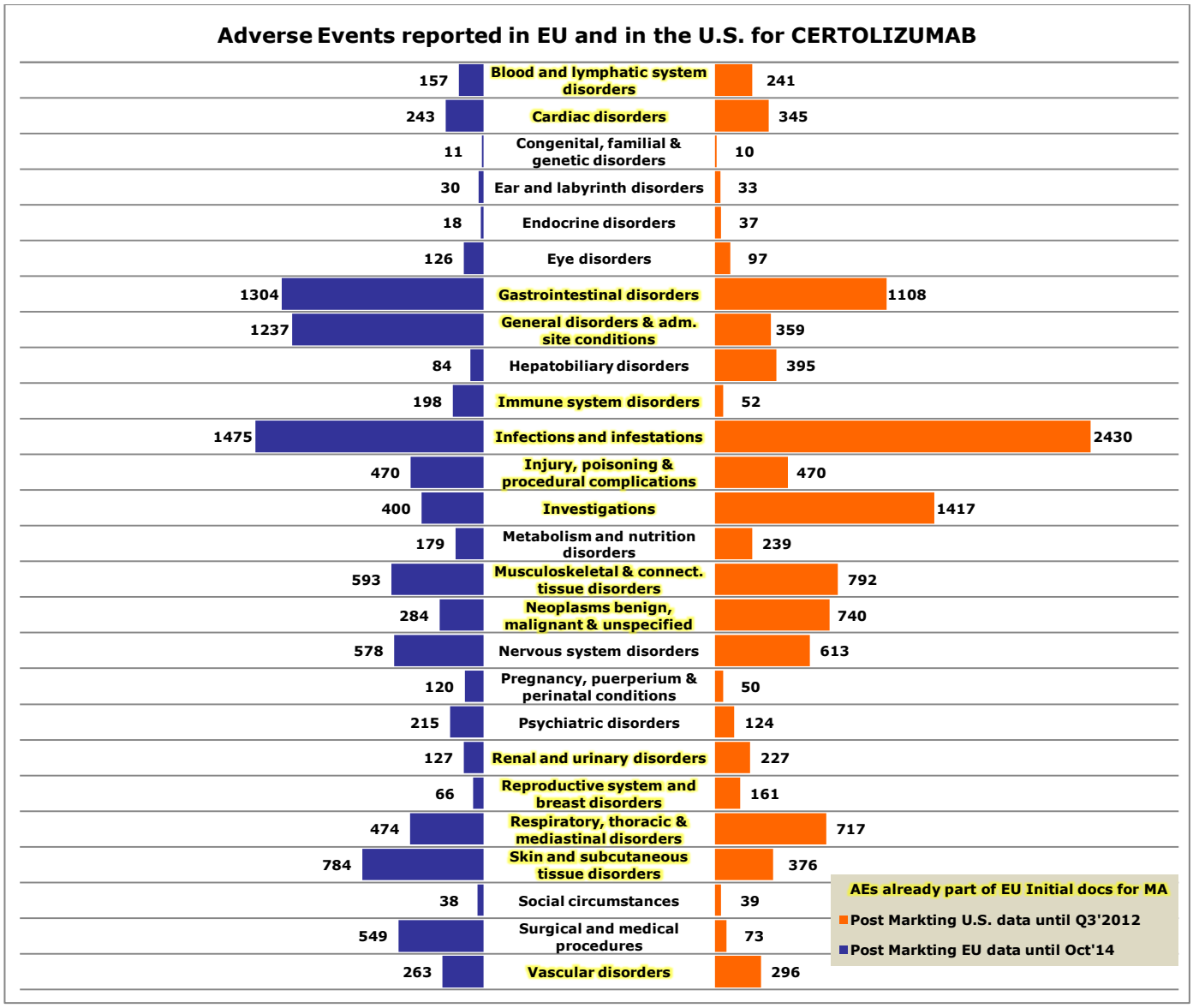

Chart 12 – Individual cases sorted by reactions groups (MedDRA 17.1 SOC), submitted for CERTOLIZUMAB PEGOL prior and post MA in Europe and post MA the United States

Comments to CERTOLIZUMAB PEGOL cumulative chart:

1. SOCs included in EU pre-MA reports vs SOCs included in EU post-MA reports

Main discrepancies: There are cases of SOCs with a considerable number of AE reports (>150) in post-MA and which were not foreseen by pre-MA clinical trials data. These include*: Metabolism and nutrition disorders*; *Nervous system disorders*; *Psychiatric disorders* & *Surgical and Medical Procedures*.

2. SOCs included in EU post-MA reports vs. SOCs included in U.S. post-MA reports

Certolizumab pegol is approved in EU and in the U.S. for different indications (with the exception of Rheumatoid Arthritis which is common), which may help to explain some significant discrepancies in the numbers above observed.

Main discrepancies: Having EU data as reference, there were some U.S. bars which were expected to be bigger but are not: these include *General disorders and administration site conditions*; *Psychiatric disorder*; *Skin and subcutaneous tissue disorders* & *Surgical and Medical Procedures*.

Based on EU data, there were also some U.S. bars which were expected to be smaller: *Hepatobiliary disorders*; *Investigations* & *Neoplasms benign, malignant and unspecified (including cysts and polyps)*.

2. Comparison of AEs reported for anti-TNFα mAbs

In the previous analysis of the AEs reported for each anti-TNFα mAb it was visible that there were some discrepancies in the AEs reported pre and post MA as well as some discrepancies in AEs reported in Europe and the United States.

Following those mAb-specific analysis, it becomes important to see how the AEs are being reported throughout anti-TNFα mAbs class.

The table below intends to provide a global picture on the ranking of mostly reported AEs for anti-TNFα mAbs. For comparison purposes, there was the need to choose a common data set and, for that reason, the table only comprises the EU reports post-MA.

Table 15 – Comparison of most commonly reported AEs for anti-TNFα mAbs (post-marketing EU data)

MedDRA 17.1 System Organ Class (SOC)	Post-Marketing EU data – ranking of most reported AEs				Comments
	ADALIMUMAB	INFLIXIMAB	GOLIMUMAB	CERTOLIZUMAB	
Gastrointestinal disorders	3rd	3rd	6th	2nd	Transversally reported AEs for all anti- TNFα mAbs.
General disorders and administration site conditions	2nd	2nd	2nd	3rd	Most reported AEs for all anti-TNFα mAbs.
Infections and Infestations	1st	1st	1st	1st	Most reported AEs for all anti-TNFα mAbs.
Injury, poisoning and procedural complications	10th	4th	8th	9th	Transversally reported AEs for all anti-TNFα mAbs.
Investigations	9th	10th	10th	10th	Least reported AEs for all anti-TNFα mAbs.
Musculoskeletal and connective tissue disorders	5th	8th	5th	5th	Transversally reported AEs for all anti-TNFα mAbs.
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	4th	9th	9th	--	
Nervous system disorders	6th	7th	3rd	6th	Transversally reported AEs for all anti-TNFα mAbs.
Respiratory, thoracic and mediastinal disorders	8th	5th	7th	8th	Transversally reported AEs for all anti-TNFα mAbs.
Skin and subcutaneous tissue disorders	7th	6th	4th	4th	Transversally reported AEs for all anti-TNFα mAbs.
Surgical and medical procedures	--	--	--	7th	

NOTE: The colours represent the ranking of reported AEs. **GREEN** represents the mostly reported AEs whereas **RED** the least reported AEs.

According to the above table, anti-TNFα mAbs seem to have a very similar profile of AEs reported in EU following MA. There are only 2 out of 11 (≈18%) SOCs above listed which are not transversally applicable to all mAbs of this class: *Neoplasms benign, malignant and unspecified (incl cysts and polyps)* & *Surgical and medical procedures*. This means that 82% of profile is similar in terms of the AEs mostly reported. Of course each mAb may have different rankings of AEs reported. Still, in general, the profiles seems constant throught all anti-TNFα mAbs – which represents great figures for similarity through this mAbs class.

Although the mAbs comprised in anti-TNFα class have some differences, including the type of mAb (2 human, 1 humanised and 1 chimeric mAb) and the configuration of mAbs (1 mAb is a

pegylated humanised Fab' fragment), it is interesting to observe that those differences did not affect significantly the general outcome of AEs reported to each mAb.

Similar treatment indication and administration routes may have been a key factor for the consistency of AEs observed throughout the anti-TNFα mAbs class. These similarities are aligned with the concept of AEs being correlated to mAbs mechanism of action.

For the particular case of anti-TNFα mAbs, the mostly reported AEs were i) *Infections and Infestations*, ii) *General disorders and administration site conditions* as well as iii) *Gastrointestinal disorders* and all these are according to the expectation.

While *General disorders and administration site conditions* are antibody-related AEs which generally occur for every mAb; *Infections and Infestations* & *Gastrointestinal disorders* can be correlated to the specific Mechanism of Action of anti-TNFα.

As immunosuppressors, it is expected that anti-TNFα mAbs decrease the immune system capabilities and raise the chances for *Infections and Infestations* to arise.

On what regards *Gastrointestinal disorders*, it is interesting to observe that, similar to anti inflammatory drugs, these mAbs also have this kind of AEs.

TNFα has been shown to stimulate prostaglandin E2 synthesis [62] and these prostaglandins E2, in turn, have been shown to have strong cytoprotective effects on the gastric mucosa [87]. It is expected that anti-TNFα mAbs alter the usual TNFα-mediated effects and these can include the usual Prostaglandin E2 gastro protective effect. It is therefore possible that inhibition of TNFα can lead to a greater number of *Gastrointestinal disorders*.

I. Anti-VEGF mAbs

Most approved and experimental anticancer antibodies directly target tumour cells. Alternative strategies include inhibiting angiogenesis or directly targeting tumour neovasculature. Angiogenesis (blood vessel neoformation) is a tightly regulated process responsible for the development of new blood vessels from a pre-existing vascular network. During development and normal physiological processes such as wound healing and the menstrual cycle, angiogenesis is regulated by endogenous activators and inhibitors [38, 88-91].

In pathological settings, such as age-related macular degeneration, rheumatoid arthritis, diabetic retinopathy and tumour growth and metastasis, angiogenesis is critical for disease progression. An independent blood supply is critical for a tumour to grow beyond a certain size and spread (metastasise) to other parts of the body. Tumours develop their own blood supply by releasing vascular endothelial growth factor (VEGF) – a key driver for tumour growth. The neovascularisation enhanced by the release of VEGF allows the tumour cells to acquire a growth advantage and proliferative autonomy compared to the normal cells [38, 88-91].

As the vascular endothelial growth factor (VEGF) family of proteins are key regulators tumour angiogenesis, they provide attractive targets for anti-cancer therapies [91]. Targeting the tumour vasculature has several potential advantages over direct tumour targeting: the vasculature is more accessible to antibodies; vasculature damage has a multiplicative effect as many tumour cells are dependent on each capillary; and as vascular endothelial cells are not transformed, they seem less likely to become resistant to antibody therapy [38].

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen and survival factor as well as regulator and promoter of angiogenesis during developmental, physiological, and pathological processes. It encompasses a class of proteins: VEGF-A, B, C, D, and placental growth factor, among which VEGF-A is the most relevant for angiogenesis and vascular permeability [90-93].

Apart from VEGF physiological functions it has a decisive role in tumour-angiogenesis and in the pathogenesis of neovascular eye diseases, including neovascular AMD, diabetic retinopathy, and retinopathy of prematurity. Endothelial cells that are present in choroidal neovascular membrane are strongly dependent on VEGF for cell survival and interference with VEGF activity induces endothelial apoptosis. It is this difference between normal and choroidal neovascularisation (CNV)-associated vasculature with respect to VEGF dependence by endothelial cells for survival that support the rationale for clinical use of an anti-VEGF Fab as therapeutic agent to treat patients with age-related macular degeneration (AMD) [90-93].

In animal models, murine mAb VEGF A4.6.1. revealed to be a high affinity mAb capable of recognizing all VEGF isoforms, which would inhibit the growth of a variety of human tumour cell lines in nude mice and inhibit the iris neovascularisation secondary to retinal ischemia in a primate model following intraocular administration. Murine antibodies have, however, a major limitation in what regards the potential risk of an immune reaction to occur; a powerful

approach to overcome this limitation in the clinical use of monoclonal antibodies is "humanization" of the murine antibodies. In the case of murine VEGF mAb, the humanisation resulted in a mAb which exhibited only a slight reduction in binding compared to the parent murine antibody, but this modest reduction in on-rate did not result in any decreased ability to antagonize VEGF bioactivity and both antibodies (fully murine & humanised) had essentially identical activity, both in an endothelial cell proliferation assay and in an *in vivo* tumour model [90].

Both Ranibizumab (LUCENTIS) and Bevacizumab (AVASTIN) were derived from the murine monoclonal antibody A4.6.1. Ranibizumab was developed from a humanised Fab variant of A4.6.1, known as MB1.6. This Fab then underwent a series of modifications: Affinity selection using phage display technology increased the affinity of Ranibizumab for VEGF-A by several times. Thus, Ranibizumab cannot simply be described as a Fab of Bevacizumab because their complementarity-determining regions (CDR) are markedly different [92].

In contrast to a full-size antibody, Ranibizumab cannot bind complement because it lacks the Fc (Fragment crystallisable) region. The increased potency, the smaller molecular size compared to a full-length antibody for enhanced penetration into the retina and choroid, and the lack of the Fc region were considered to be advantageous for intravitreal efficacy [92].

When comparing both mAbs [92]:

- ↑Bevacizumab – the systemic half-life of Ranibizumab is a few hours compared to roughly 3 weeks for Bevacizumab. This may be a disadvantage in respect to the number of required re-injections;
- ↑Ranibizumab – the affinity of Ranibizumab for VEGF-A is higher than that of Bevacizumab but it is unclear whether this has significant clinical implications;
- ↑Ranibizumab – the presence of the Fc fragment in Bevacizumab might turn the patients receiving Bevacizumab more susceptible to the development of an immune response to the agent.

1. mAbs targeted for VEGF

Table 16 – anti-VEGF mAbs approved indications for Europe and for the United States

Anti-VEGF mAbs				
BEVACIZUMAB		**RANIBIZUMAB**		**Indications**
U.S.	**EU**	**U.S.**	**EU**	
X	X			*Metastatic Carcinoma of the Colon or Rectum (mCRC)*
	X			*Metastatic breast cancer (mBC)*
X	X			*Non-Small Cell Lung Cancer*
X	X			*Metastatic Renal Cell Carcinoma (mRCC)*
	X			*Epithelial ovarian, fallopian tube and primary peritoneal cancer*
X				*Glioblastoma*
X				*Cervical Cancer*
		X	X	*Age-related macular degeneration (AMD)*
		X	X	*Diabetic macular oedema (DME)*
		X	X	*Macular oedema secondary to Retinal Vein Occlusion (RVO)*
			X	*Choroidal Neovascularisation (CNV) Secondary to Pathologic Myopia*

1.1. BEVACIZUMAB

Bevacizumab (trade name AVASTIN) is currently in the market with different indications for Europe and for the United Sates.

Europe and the United States share the approval of Bevacizumab treatment for colorectal cancer, non-small cell lung cancer and kidney cancer. However, other Bevacizumab's indications remain different in Europe and the United States.

Currently only Europe has the Bevacizumab approval granted for treatment of advanced stages of breast cancer and ovarian cancer. For a while Bevacizumab was also approved in the United States for the treatment of breast cancer (approval granted in 2008 through the US FDAs Accelerated Approval Program) but following 3 years in the market this indication was revoked (2011) after concluding that the drug has not been shown to be safe and effective for the treatment of breast cancer (revocation was announced in November 2011 by the US FDA Commissioner Margaret Hamburg) [94].

The United States, on another hand, have Bevacizumab also approved for treatment of patients with progressive Glioblastoma following prior therapy whereas Europe has not. Europe refused the Glioblastoma indication on the grounds that effectiveness of Bevacizumab in combination with radiotherapy and temozolomide had not been sufficiently demonstrated and that there was no benefit in terms of overall survival [88, 95, 96].

The US FDA and EMEA have reached different scientific conclusions on the efficacy and safety of drugs in the past. The reasons for different decisions are not readily apparent between the US FDA and EMEA [94].

1.1.1. BEVACIZUMAB Characterization

Pharmacotherapeutic group: Monoclonal antibodies
ATC code: L01XC07 [57]

L — ANTINEOPLASTIC AND IMMUNOMODULATING AGENTS
L01 — ANTINEOPLASTIC AGENTS
L01X — OTHER ANTINEOPLASTIC AGENTS
L01XC — Monoclonal antibodies

Bevacizumab (AVASTIN) is a full-size recombinant humanised monoclonal IgG1 antibody (93% human, 7% murine sequences) that binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF) *in vitro* and *in vivo* assay systems. Neutralisation of VEGF's biologic activity is achieved through a steric blocking of the binding of VEGF to its receptors Flt-1 (VEGFR-1) and KDR (VEGFR-2) on the surface of endothelial cells. Receptors activation normally induces their tyrosine phosporylation and the subsequent series of signal transduction events elicit mitogenic and pro-survival activity signals for the vascular endothelial cells.

Bevacizumab is produced by DNA technology in Chinese Hamster Ovary cells and it contains human framework regions in addition to the complementarity-determining regions of a murine antibody which bind to VEGF.

The molecular weight of Bevacizumab is approximately 149 kD [92, 97-99].

1.1.2. BEVACIZUMAB Mechanism of Action

Bevacizumab is a tumour-starving (anti-angiogenic) therapy. Bevacizumab is designed to bind to vascular endothelial growth factor (VEGF), the key driver of vasculogenesis and prevent VEGF interaction to its receptors – Flt-1 (VEGFR-1) and KDR (VEGFR-2) – on the surface of endothelial cells.

The interaction of VEGF with its receptors leads to endothelial cell proliferation and new blood vessel formation in *in vitro models* of angiogenesis. Therefore, neutralising the biological activity of VEGF results in regression of tumours vascularisation, normalisation of the remaining tumour vasculature, and inhibition of the formation of new tumour vasculature, thereby inhibiting tumour growth[97, 98, 100].

1.1.3. BEVACIZUMAB Treatment

Table 17 – Bevacizumab Treatment: Method of administration and Indications

BEVACIZUMAB | *Administration:* intravenous infusion					
U.S.	**EU**	**Indications**	**Population**	**Indications Details**	**References**
X	X	***Metastatic Carcinoma of the Colon or Rectum (mCRC)***	Adults	– in combination with fluoropyrimidine-irinotecan or fluoropyrimidine-oxaliplatin-based chemotherapy is indicated for second-line treatment of metastatic colorectal cancer which has progressed on a first-line Bevacizumab-containing regimen	[97, 98, 101]
	X	***Metastatic breast cancer (mBC)***	Adults	– in combination with paclitaxel is indicated for first-line treatment of metastatic breast cancer – in combination with capecitabine is indicated for first-line treatment of metastatic breast cancer which has not responded adequately to other chemotherapy options, including taxanes or anthracyclines	[98]
X	X	***Non-Small Cell Lung Cancer***	Adults	– in addition to platinum-based chemotherapy, is indicated for first-line treatment of unresectable advanced, metastatic or recurrent non-small cell lung cancer other than predominantly squamous cell histology	[97, 98, 101]
X	X	***Metastatic Renal Cell Carcinoma (mRCC)***	Adults	– in combination with interferon alfa-2a is indicated for first line treatment of advanced and/or metastatic renal cell cancer	[97, 98]
	X	***Epithelial ovarian, fallopian tube and primary peritoneal cancer***	Adults	– in combination with carboplatin and paclitaxel is indicated for the front-line treatment of advanced stages epithelial ovarian, fallopian tube, or primary peritoneal cancer – in combination with carboplatin and gemcitabine, is indicated for treatment of first recurrence of platinum-sensitive epithelial ovarian, fallopian tube or primary peritoneal cancer which has not received prior therapy with a VEGF inhibitor/receptor–targeted agent – in combination with paclitaxel, topotecan, or pegylated liposomal doxorubicin is indicated for the treatment of adult patients with platinum-resistant recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer which received no more than 2 prior chemotherapy regimens and which has not received prior therapy with a VEGF inhibitor/receptor–targeted agent	[98]
X		***Glioblastoma***	Adults	– treatment of Glioblastoma with progressive disease following prior therapy as a single agent	[97]
X		***Cervical Cancer***	Adults	– in combination with paclitaxel and cisplatin or paclitaxel and topotecan for the treatment of persistent, recurrent, or metastatic carcinoma of the cervix	[97]

1.1.4. BEVACIZUMAB Adverse Events (as per EU Initial MA documents)

Table 18 – Bevacizumab Adverse Events as per European Initial Marketing-authorisation Documents

MedDRA 17.1 System Organ Class (SOC)	AEs Detailed	References
Cardiac disorders	– Congestive heart failure (CHF)/cardiomyopathy – these events varied in severity from asymptomatic declines in left ventricular ejection fraction to symptomatic CHF requiring hospitalisation and treatment	[99]
Gastrointestinal disorders	– Diarrhoea – Gastrointestinal perforation – Bevacizumab has been associated with serious cases of gastrointestinal. The common feature among these cases was intra-abdominal inflammation, either from gastric ulcer disease, tumour necrosis, diverticulitis, or chemotherapy-associated colitis	[99]
Injury, poisoning and procedural complications	– Wound healing – post-operative bleeding or wound healing complication were observed in some Bevacizumab-treated patients who underwent major surgery while receiving treatment	[99]
Investigations	– Blood alkaline phosphatase increased – Blood glucose increased – Blood phosphorus decreased – Blood potassium decreased – Neutrophil count decreased – Presence of urine protein – White blood cell count decreased	[99]
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	– Tumour-associated haemorrhage – the haemorrhagic events that have been observed in clinical studies were predominantly tumour-associated haemorrhage	[99]
Renal and urinary disorders	– Proteinuria – it ranged in severity from clinically asymptomatic, transient, trace proteinuria to nephritic syndrome, with the great majority as grade 1 proteinuria	[99]
Vascular disorders	– Deep thrombophlebitis – Haemorrhage – grade 3 and 4 bleeding events were reported in some Bevacizumab-treated patients – Hypertension – grade 3 hypertension (requiring oral anti-hypertensive medication) was observed in some Bevacizumab-treated patients – Thromboembolism – thromboembolic events including CVAS, MIS, TIAS, and other arterial thromboembolic events was higher in some Bevacizumab-treated patients	[99]

1.1.5. BEVACIZUMAB Adverse Events Charts (following MA)

- ***European Data (EU database of suspected adverse drug reactions reports)***

The data here displayed was launched by EMA in the European database of suspected adverse drug reactions reports. Reports included in this database correspond to the data submitted electronically to EudraVigilance by national medicines regulatory authorities and by pharmaceutical companies that hold marketing authorisations (licences) for the medicines.

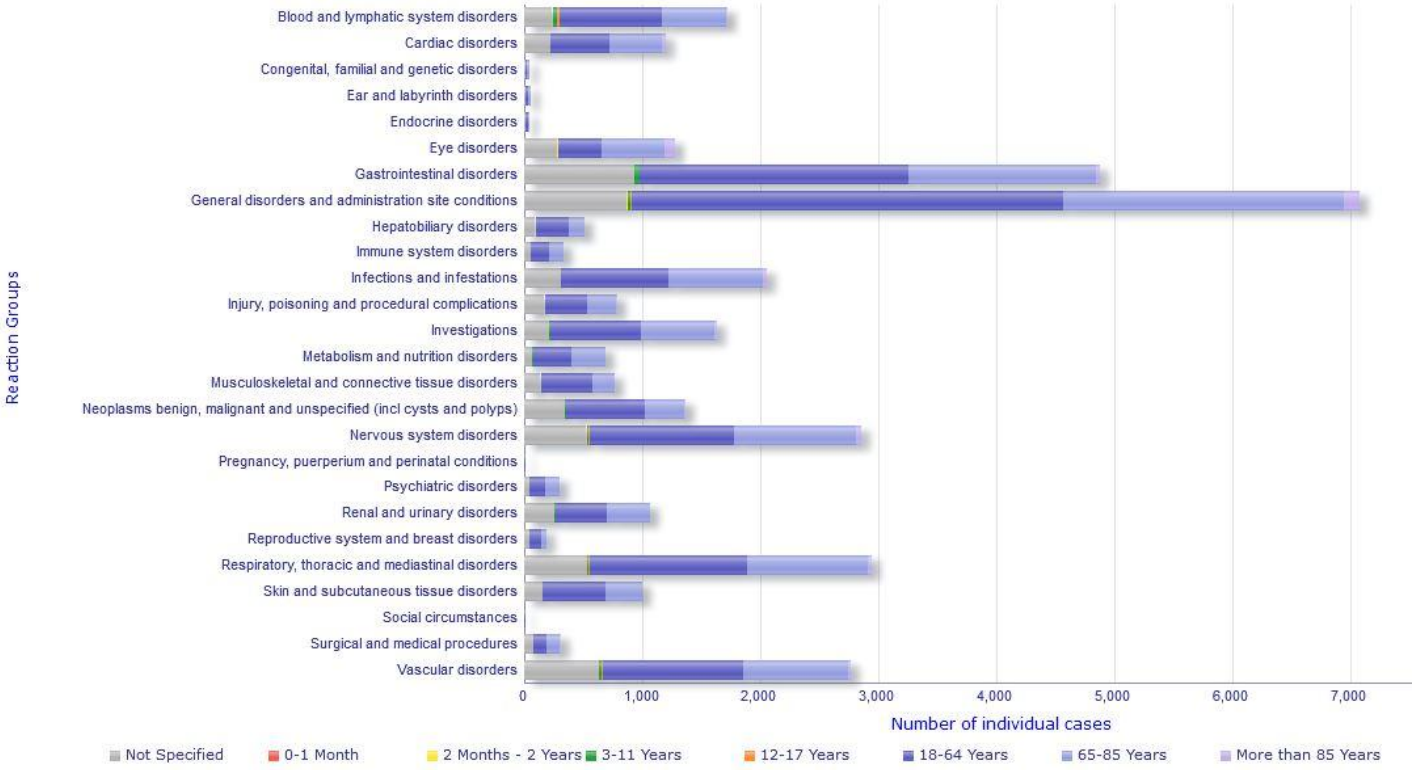

Chart 13 – Individual cases sorted by reactions groups, submitted for BEVACIZUMAB (up to Oct'14)
***in* European database of suspected adverse drug reaction reports (www.adrreports.eu)**
(data extracted on 26-Nov-2014)

The chart above displays the number of cases reported for each reaction group, divided by age. The original chart (online format) enabled to pass the mouse through each bar and reveal the number of cases for a specific age. Below are the values of the biggest bars.

EU Top 10 of Individual Cases (by reactions groups):

1. General disordes and administration site conditions (7078 cases)
2. Gastrointestinal disorders (4874 cases)
3. Respiratory, thoracic and mediastinal disorders (2942 cases)
4. Nervous system disorders (2860 cases)
5. Vascular disorders (2764 cases)
6. Infections and Infestations (2055 cases)
7. Blood and lymphatic system disorders (1722 cases)
8. Investigations (e.g. Chest X-ray abnormal; Electocardiogram QT prolonged) (1634 cases)
9. Neoplasms benign, malignant and unspecified (incl cysts and polyps) (1369 cases)
10. Eye disorders (1280 cases)

- ***FDA Adverse Event Reporting System (FAERS) Data (DrugCite.com)***

The data here displayed was made available by DrugCite database. Results included in this database correspond to the collection and analysis of reported adverse performed by FDA Adverse Events Reporting System (FAERS). These reports are submitted by physicians, healthcare consumers, lawyers amongst others.

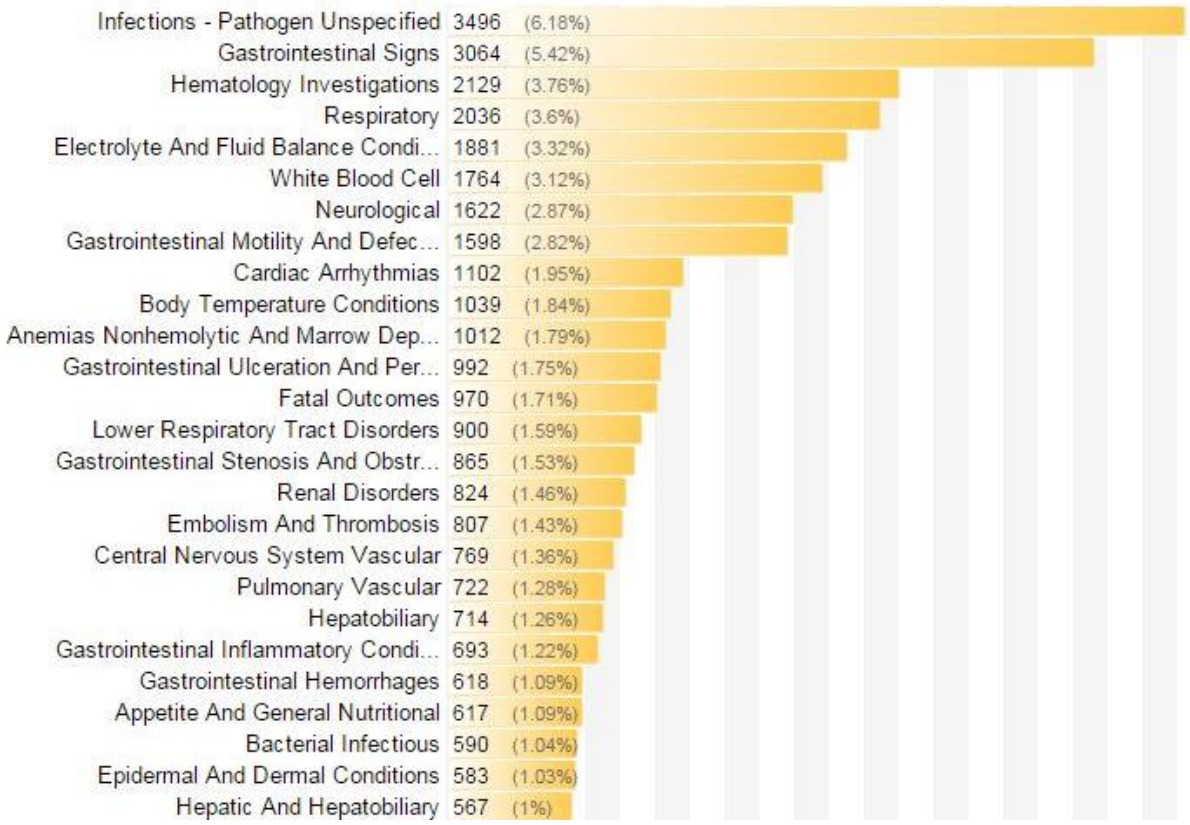

Chart 14 – Individual cases sorted by reactions groups, submitted for BEVACIZUMAB and other drugs with same active substance (Q1 2004 – Q3 2012) *in* DrugCite.com (www.drugcite.com)

(data extracted on 25-Nov-2014)

The chart above displays, for each reaction group, the number of cases reported where the drug was flagged as a suspect drug causing the adverse event. All percentages (%) are the cases reported for a specific reaction group within the sum up of all reported cases in all reactions groups (percent does not represent rate related to drug utilization).

U.S. Top 10 of Individual Cases (by reactions groups):

1. Infections – Pathogen Unspecified (3496 cases)
2. Gastrointestinal Signs (3064 cases)
3. Hemathology Investigations (2129 cases)
4. Respiratory (2036 cases)
5. Electrolyte And Fluid Balance Conditions (1881 cases)
6. White Blood Cell (1764 cases)
7. Neurological (1622 cases)
8. Gastrointestinal Motility And Defecation Conditions (1598 cases)
9. Cardiac Arrhythmias (1102 cases)
10. Body Temperature Conditions (1039 cases)

- ***Comparison of Adverse Events reported in EU and in the U.S.***

Following the analysis of the Adverse Events previously presented, it became clear that there were some differences between EU data and U.S. data. In order to have a visually enhanced perception of such differences, it was decided to create a cumulative chart which would aggregate all the data previously presented:

- Post Marketing EU data (Blue bars in the cumulative chart)
- Post Marketing U.S. data (Orange bars in the cumulative chart)
- EU data as per Initial documents for MA (MedDRA SOCs highlighted in yellow in the cumulative chart)

For aggregation purposes, a common classification had to be used and so MedDRA SOC was the selected classification for the cumulative chart below presented.

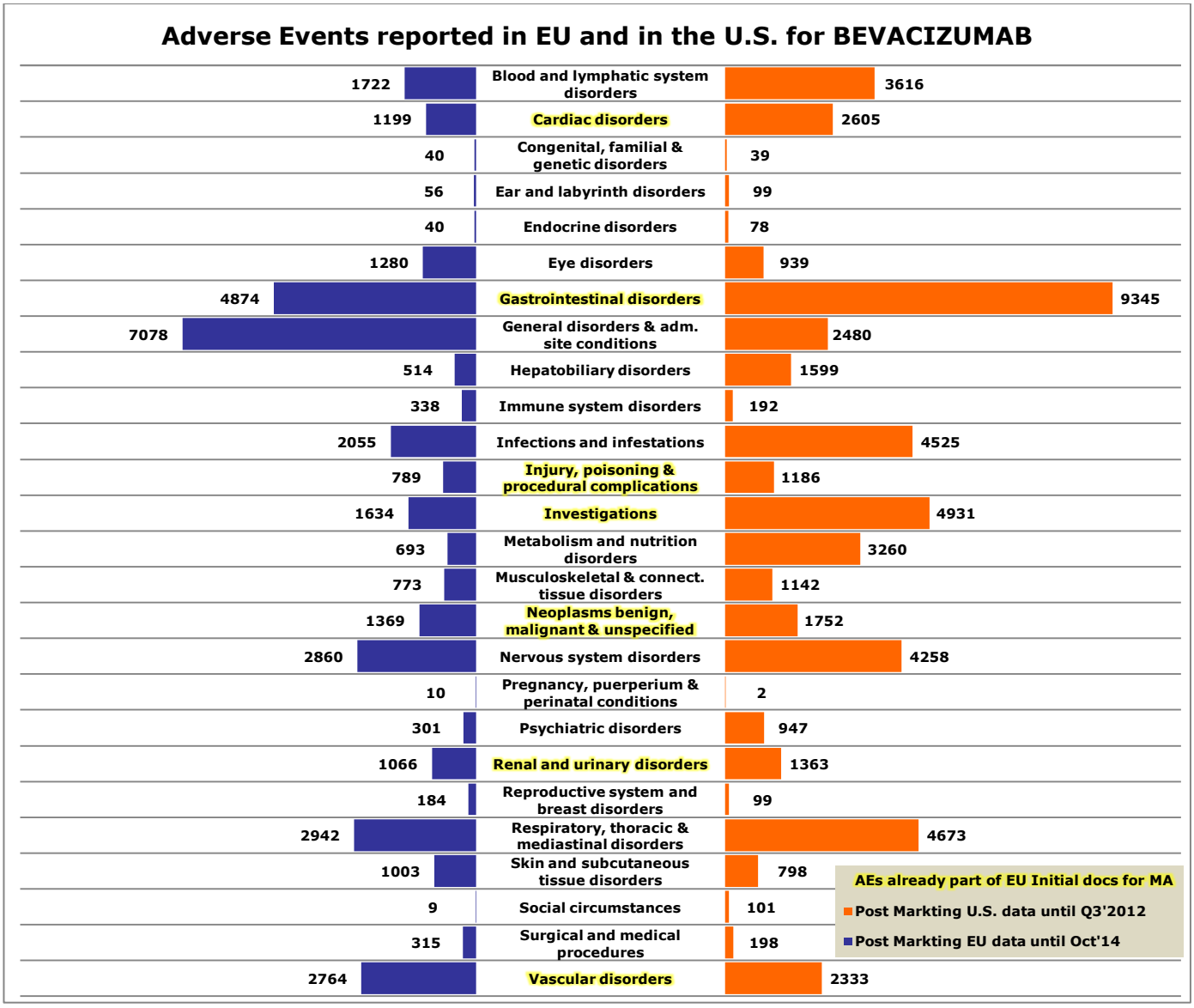

Chart 15 – Individual cases sorted by reactions groups (MedDRA 17.1 SOC), submitted for BEVACIZUMAB prior and post MA in Europe and post MA the United States

Comments to BEVACIZUMAB cumulative chart:

1. SOCs included in EU pre-MA reports vs SOCs included in EU post-MA reports

Main discrepancies: There are cases of SOCs with a considerable number of AE reports (>1000) in post-MA and which were not foreseen by pre-MA clinical trials data. These include*: Blood and lymphatic system disorders*; *Eye disorders*; *Infections and infestations*; *Nervous system disorders*; *Respiratory, thoracic and mediastinal disorders* & *Skin and subcutaneous tissue disorders*.

2. SOCs included in EU post-MA reports vs. SOCs included in U.S. post-MA reports

Although there are some discrepancies in the main SOCs referred on EU post-MA reports and U.S. post-MA reports and these are not very relevant in the global outcome of the chart.

Small discrepancies: Having EU data as reference, there were some U.S. bars which were expected to be bigger but are not: these include *Eye disorders*; *General disorders and administration site conditions.*

In general, it was observed that Bevacizumab had a similar profile of AEs reported post-MA in EU and in the U.S.

1.2. RANIBIZUMAB

Ranibizumab (trade name LUCENTIS) is currently in the market with slightly different indications for Europe and for the United Sates.

Ranibizumab was jointly developed by Genentech and Novartis. Genentech has the commercial rights in the US, while Novartis has exclusive rights in the rest of the world. Lucentis is a registered trademark of Genentech Inc [102, 103].

Europe and the United States share the approval of Ranibizumab treatment for wet age-related macular degeneration (wet AMD), diabetic macular oedema (DME) and macular oedema following retinal vein occlusion (RVO) [104]. Additionally, in Europe, Novartis has been granted approval by the European Commission for Ranibizumab to treat patients with visual impairment due to choroidal neovascularisation (CNV) secondary to pathologic myopia (myopic CNV) [102-105].

1.2.1. RANIBIZUMAB Characterization

Pharmacotherapeutic group: Ophthalmologicals
ATC code: S01LA04 [57]
S — SENSORY ORGANS
S01 — OPHTHALMOLOGICALS
S01L — OCULAR VASCULAR DISORDER AGENTS
S01LA — Antineovascularisation agents

Ranibizumab (LUCENTIS) is a recombinant humanised monoclonal antibody fragment (Fab) produced in *Escherichia coli* cells, by standard recombinant DNA technology, and is targeted against all biologically active forms of human vascular endothelial cell growth factor-A (VEGF-A) [92, 103, 106, 107].

Increased levels of VEGF-A are seen in wet AMD and other ocular diseases such as diabetic macular oedema (DME) and retinal vein occlusion (RVO). Ranibizumab was specifically designed for the eye, minimizing systemic exposure [103].

Ranibizumab, which lacks an Fc region, is produced by an E. coli expression system by recombinant DNA technology. It has a molecular weight of approximately 48 kDa [106, 108, 109].

1.2.2. RANIBIZUMAB Mechanism of Action

Ranibizumab binds with high affinity to the VEGF-A isoforms (e.g. VEGF110, VEGF121 and VEGF165), thereby preventing binding of VEGF-A to its receptors VEGFR-1 and VEGFR-2, on the surface of endothelial cells.

Usually VEGF-A binding to its receptors leads to endothelial cell proliferation and neovascularisation, as well as vascular leakage, all of which are thought to contribute to the progression of the neovascular form of age-related macular degeneration (AMD), pathologic myopia or to visual impairment caused by either diabetic macular oedema (DME) or macular oedema secondary to retinal vein occlusion (RVO). Ranibizumab binding to VEGF-A prevents the interaction with the receptors; therefore reducing endothelial cell proliferation, vascular leakage, and new blood vessel formation [93, 105, 106, 108, 109].

1.2.3. RANIBIZUMAB Treatment

Table 19 – Bevacizumab Treatment: Method of administration and Indications

BEVACIZUMAB | *Administration:* intravitreal injections					
U.S.	**EU**	**Indications**	**Population**	**Indications Details**	**References**
X	X	***Age-related macular degeneration (AMD)***	Adults	– treatment of neovascular (wet) age-related macular degeneration (AMD), until maximum visual acuity is achieved (i.e. the patient's visual acuity is stable for 3 consecutive monthly assessments)	[97, 108]
X	X	***Diabetic macular oedema (DME)***	Adults	– treatment of visual impairment due to diabetic macular oedema (DME), until maximum visual acuity is achieved (i.e. the patient's visual acuity is stable for 3 consecutive monthly assessments)	[97, 108]
X	X	***Macular oedema secondary to Retinal Vein Occlusion (RVO)***	Adults	– treatment of visual impairment due to macular oedema secondary to retinal vein occlusion (branch RVO or central RVO), until maximum visual acuity is achieved (i.e. the patient's visual acuity is stable for 3 consecutive monthly assessments)	[97, 108]
	X	***Choroidal Neovascularisation (CNV) Secondary to Pathologic Myopia***	Adults	– treatment of visual impairment due to choroidal neovascularisation (CNV) secondary to pathologic myopia (PM)	[108]

1.1.1. RANIBIZUMAB Adverse Events (as per EU Initial MA documents)

Table 20 – Ranibizumab Adverse Events as per European Initial Marketing-authorisation Documents

MedDRA 17.1 System Organ Class (SOC)	AEs Detailed	References
Eye disorders	– Conjunctival haemorrhage – Eye pain – Foreign body sensation in eyes – Macular degeneration – Retinal detachment – Retinal haemorrhage – Retinal tear – Uveitis – Vitreous detachment – Vitreous floaters – Vitreous haemorrhage	[107]
Infections and Infestations	– Bronchitis – Endophthalmitis – Nasopharygitis – Upper respiratory tract infection	[107]
Investigations	– Intraocular pressure increased	[107]
Nervous system disorders	– Headache	[107]
Respiratory, thoracic and mediastinal disorders	– Cough	[107]
Vascular disorders	– Arterial thromboembolic events – Hypertension	[107]

1.1.1. RANIBIZUMAB Adverse Events Charts (following MA)

- ***European Data (EU database of suspected adverse drug reactions reports)***

The data here displayed was launched by EMA in the European database of suspected adverse drug reactions reports. Reports included in this database correspond to the data submitted electronically to EudraVigilance by national medicines regulatory authorities and by pharmaceutical companies that hold marketing authorisations (licences) for the medicines.

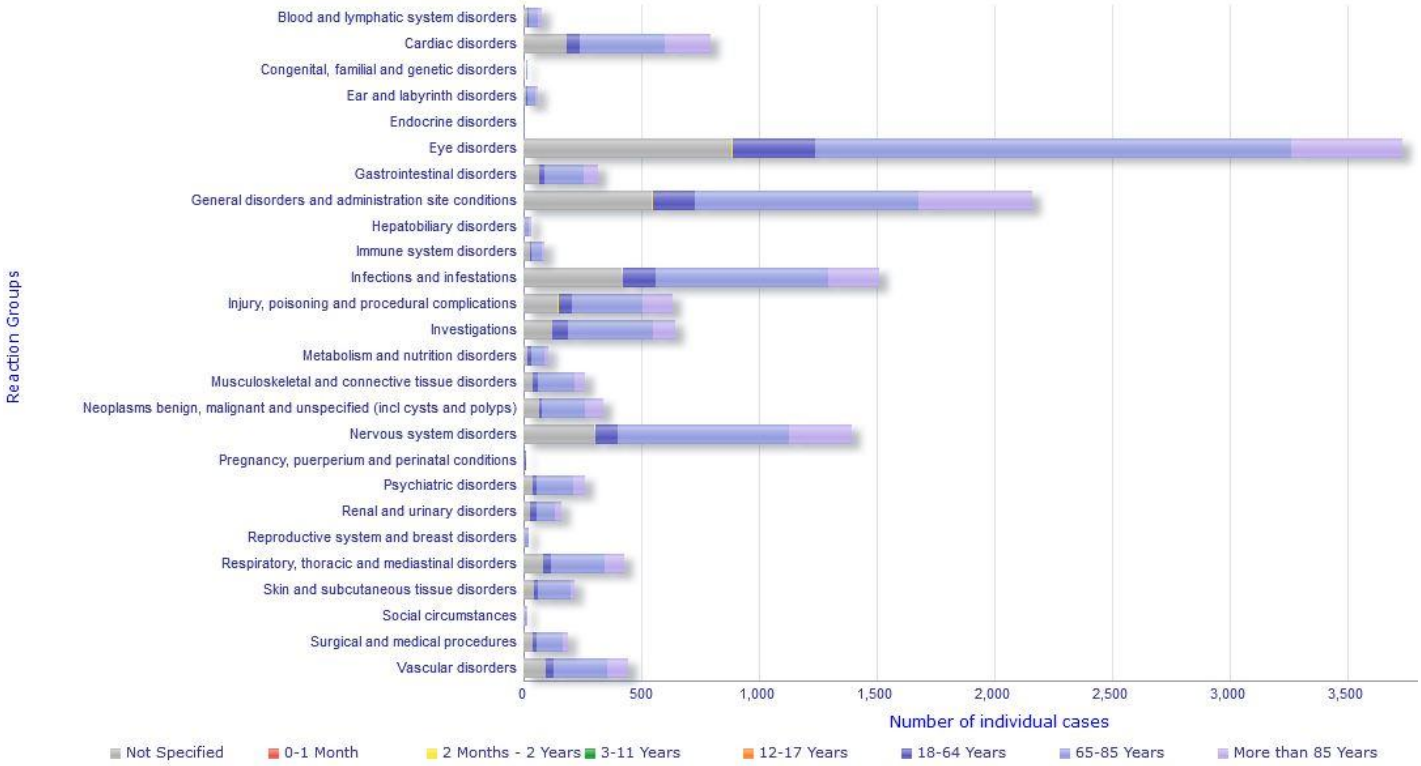

Chart 16 – Individual cases sorted by reactions groups, submitted for RANIBIZUMAB (up to Oct'14)
***in* European database of suspected adverse drug reaction reports (www.adrreports.eu)**
(data extracted on 26-Nov-2014)

The chart above displays the number of cases reported for each reaction group, divided by age. The original chart (online format) enabled to pass the mouse through each bar and reveal the number of cases for a specific age. Below are the values of the biggest bars.

EU Top 10 of Individual Cases (by reactions groups):

1. Eye disorders (3732 cases)
2. General disordes and administration site conditions (2163 cases)
3. Infections and Infestations (1512 cases)
4. Nervous system disorders (1397 cases)
5. Cardiac disorders (795 cases)
6. Investigations (e.g. Chest X-ray abnormal; Electocardiogram QT prolonged) (644 cases)
7. Injury, poisoning and procedural complications (636 cases)
8. Vascular disorders (442 cases)
9. Respiratory, thoracic and mediastinal disorders (428 cases)
10. Neoplasms benign, maligant and unspecified (incl cysts and polyps) (339 cases)

- ***FDA Adverse Event Reporting System (FAERS) Data (DrugCite.com)***

The data here displayed was made available by DrugCite database. Results included in this database correspond to the collection and analysis of reported adverse performed by FDA Adverse Events Reporting System (FAERS). These reports are submitted by physicians, healthcare consumers, lawyers amongst others.

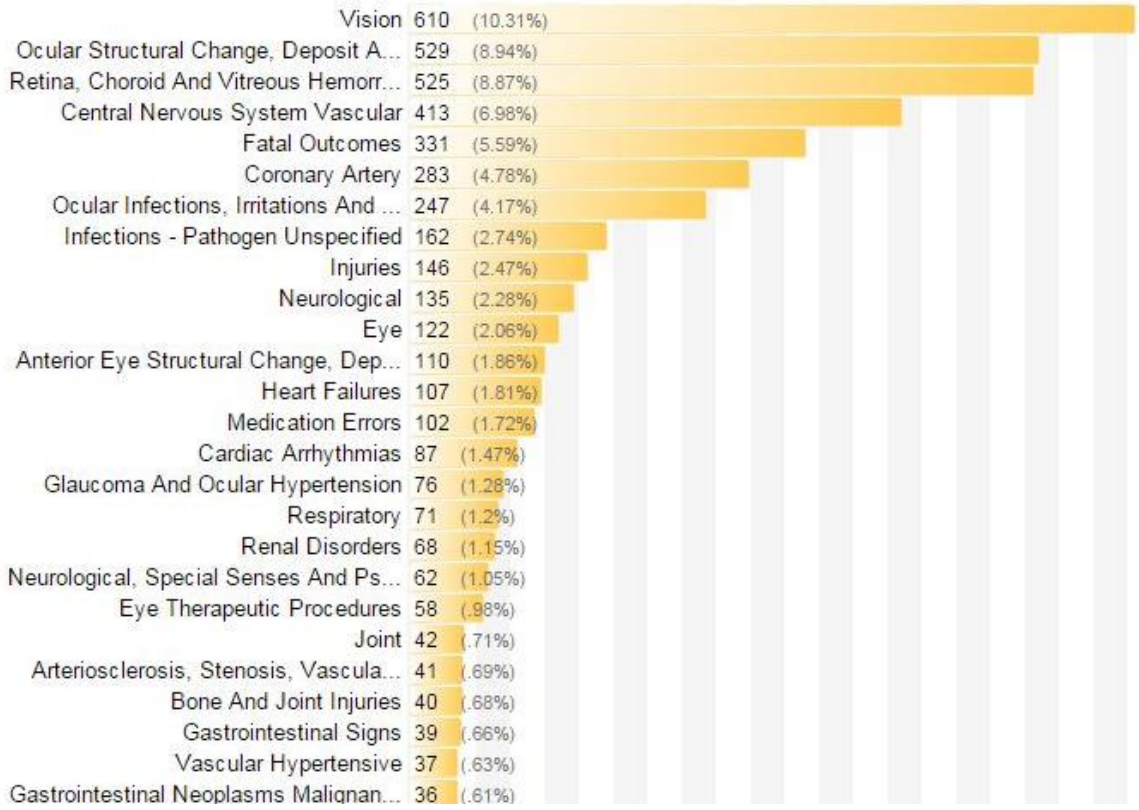

Chart 17 – Individual cases sorted by reactions groups, submitted for RANIBIZUMAB and other drugs with same active substance (Q1 2004 – Q3 2012) *in* DrugCite.com (www.drugcite.com)

(data extracted on 26-Nov-2014)

The chart above displays, for each reaction group, the number of cases reported where the drug was flagged as a suspect drug causing the adverse event. All percentages (%) are the cases reported for a specific reaction group within the sum up of all reported cases in all reactions groups (percent does not represent rate related to drug utilization).

U.S. Top 10 of Individual Cases (by reactions groups):

1. Vision (610 cases)
2. Ocular Structural Change, Deposit and Degeneration (529 cases)
3. Retina, Choroid And Vitrous Hemorrhages and Vascular (525 cases)
4. Central Nervous System Vascular (413 cases)
5. Fatal Outcomes (331 cases)
6. Coronary Artery (283 cases)
7. Ocular Infections, Irritations And Inflammations (247 cases)
8. Infections – Pathogen Unspecified (162 cases)
9. Injuries (146 cases)
10. Neurological (135 cases)

- ***Comparison of Adverse Events reported in EU and in the U.S.***

Following the analysis of the Adverse Events previously presented, it became clear that there were some differences between EU data and U.S. data. In order to have a visually enhanced perception of such differences, it was decided to create a cumulative chart which would aggregate all the data previously presented:

- Post Marketing EU data (Blue bars in the cumulative chart)
- Post Marketing U.S. data (Orange bars in the cumulative chart)
- EU data as per Initial documents for MA (MedDRA SOCs highlighted in yellow in the cumulative chart)

For aggregation purposes, a common classification had to be used and so MedDRA SOC was the selected classification for the cumulative chart below presented.

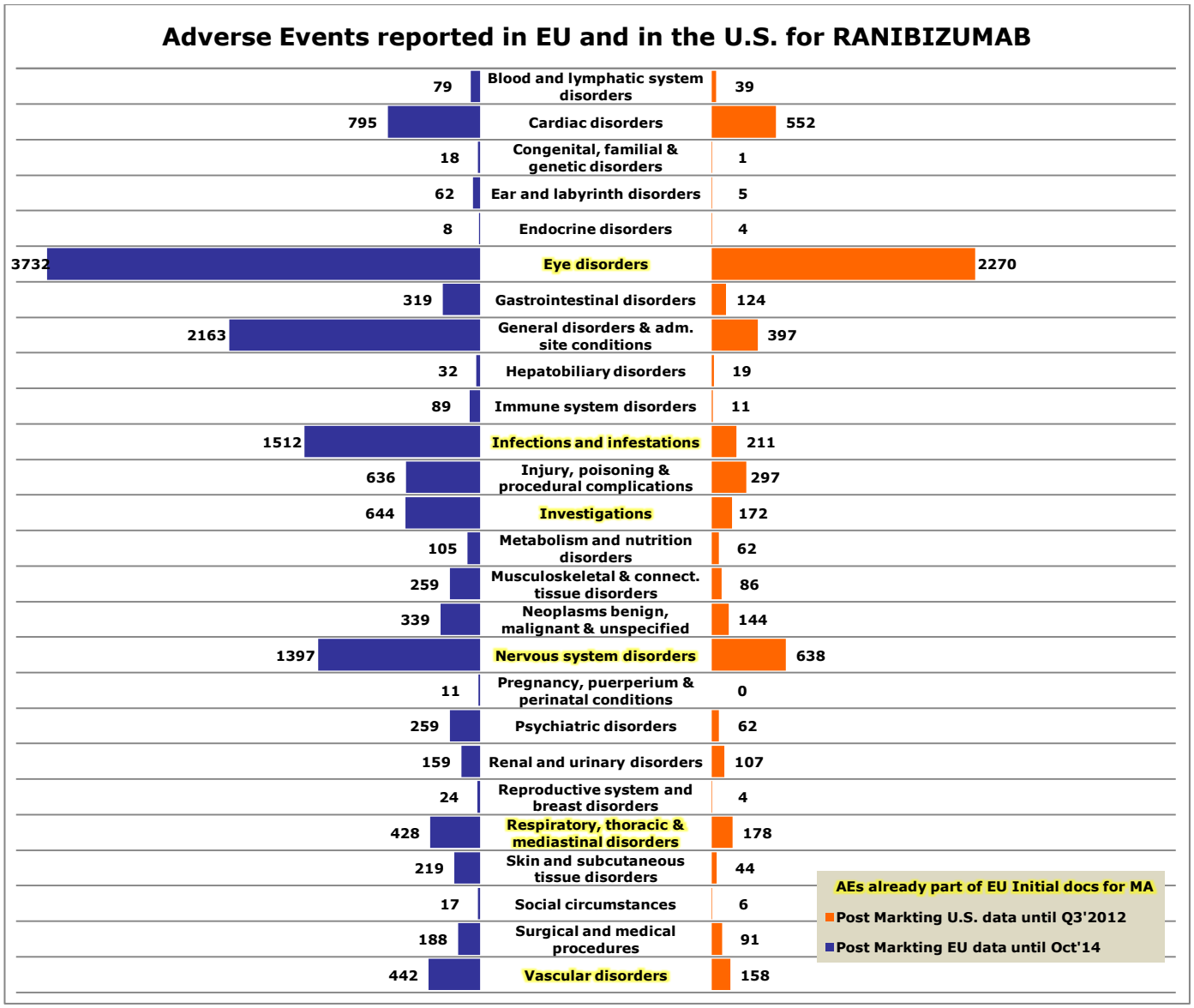

Chart 18 – Individual cases sorted by reactions groups (MedDRA 17.1 SOC), submitted for RANIBIZUMAB prior and post MA in Europe and post MA the United States

Comments to RANIBIZUMAB cumulative chart:

1. SOCs included in EU pre-MA reports vs SOCs included in EU post-MA reports

Main discrepancies: There are cases of SOCs with a considerable number of AE reports (>600) in post-MA and which were not foreseen by pre-MA clinical trials data. These include*: Cardiac disorders* & *Injury, poisoning and procedural complications*.

2. SOCs included in EU post-MA reports vs. SOCs included in U.S. post-MA reports

Although Ranibizumab is approved in EU and in the U.S. almost for the same indications, there are some significant discrepancies in the numbers above observed.

Main discrepancies: Having EU data as reference, there were some U.S. bars which were expected to be bigger but are not. These include: *General disorders and administration site conditions*; *Infections and infestations*; *Investigations*; & *Psychiatric disorders*.

2. Comparison of AEs reported for anti-VEGF mAbs

In the previous analysis of the AEs reported for each anti-VEGF mAb it was visible that there were some discrepancies in the AEs reported pre and post MA as well as some discrepancies in AEs reported in Europe and the United States.

Following those mAb-specific analysis, it becomes important to see how the AEs are being reported throught anti-VEGF mAbs class.

The table below intends to provide a global picture on the ranking of mostly reported AEs for anti-VEGF mAbs. For comparison purposes, there was the need to chose a common data set and, for that reason, the table only comprises the EU reports post-MA.

Table 21 – Comparison of most commonly reported AEs for anti-VEGF mAbs (post-marketing EU data)

MedDRA 17.1 System Organ Class (SOC)	Post-Marketing EU data – ranking of most reported AEs		Comments
	BEVACIZUMAB	RANIBIZUMAB	
Blood and lymphatic system disorders	7th	--	
Cardiac disorders	--	5th	
Gastrointestinal disorders	2nd	--	
General disorders and administration site conditions	1st	2nd	Most reported AEs for all anti-VEGF mAbs.
Eye disorders	10th	1st	Opposite profiles in these two anti-VEGF mAbs.
Infections and Infestations	6th	3rd	Transversally reported AEs for all anti-VEGF mAbs.
Injury, poisoning and procedural complications	--	7th	
Investigations	8th	6th	Transversally reported AEs for all anti-VEGF mAbs.
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	9th	10th	Least reported AEs for all anti-VEGF mAbs.
Nervous system disorders	4th	4th	Transversally reported AEs for all anti-VEGF mAbs.
Reproductive system and breast disorders	3rd	--	
Respiratory, thoracic and mediastinal disorders	--	9th	
Vascular disorders	5th	8th	Transversally reported AEs for all anti-VEGF mAbs.

NOTE: The colours represent the ranking of reported AEs. **GREEN** represents the mostly reported AEs whereas **RED** the least reported AEs.

According to the above table, anti-VEGF mAbs seem to have a quite different profile of AEs reported in EU following MA. There are 6 out of 13 (≈46%) SOCs above listed which are not transversally applicable to all this class mAbs. This means that only 54% of profile is similar in terms of AEs mostly reported.

Although the mAbs comprised in anti-VEGF class have the same mechanism of action, it is clear in this class that there are other key features that can significantly impact the outcome of AEs reported. Mechanism of Action continues to play a major role in the AEs reported but, for the particular case of anti-VEGF, there are significant differences among the mAbs of this class:

- Bevacizumab and Ranibizumab are used for different treatment indications (refer to ANNEX 1 – Underlying diseases of anti-VEGF mAbs)
- Bevacizumab and Ranibizumab administration route is completely different (intravenous infusion vs. intravitreal injection, respectively)
- Bevacizumab and Ranibizumab structure is very different (compete mAb vs. Fab fragment, respectively).

Similar to other mAbs, it is observed that anti-VEGF mAbs also have a significant number of *General disorders and administration site conditions* reported. As mentioned before, these are antibody-related AEs which generally occur for every mAb and are not class-specific AEs.

On what regards class-specific AEs, it would be expected that anti-VEGF mAbs would have effects at a vascular-level and *Vascular disorders* are in fact reported for both mAbs here comprised. The numbers of reports are different and numbers are higher for Bevacizumab, but this can be explained by the mAb structure – when compared to Ranibizumab (Fab fragment administered by intravitreal injection), Bevacizumab (complete mAb administered intravenously) has more potential for systemic reactions, including *Vascular disorders*.

The reports above observed for *Nervous system disorders* & *Cardiac disorders* may also comprise the disorders related to nervous system vasculature and cardiac vasculature and, in that case, the numbers are also predictable. Also here, the characteristics of anti-VEGF mAbs have a crucial impact on the number of reports. While *Nervous system disorders* appear for both mAbs with similar numbers, *Cardiac disorders* are very different.

This differences in *Cardiac disorders* reports may be related to the differences of these mAbs treatment indications. While Bevacizumab is used for tumours vasculature suppression, Ranibizumab is used for ocular complications. In the presence of a tumour, anti-VEGF Bevacizumab is preferably targeted to the tumour and may not have a significant cardiac impact. Ranibizumab, on another hand, is administered by intravitreal injection in patients without tumours and after administration it may have more potential to promote *Cardiac disorders*.

Gastrointestinal disorders & *Reproductive system and breast disorders* are also differently reported for the above anti-VEGF mAbs. These differences may be related to the administration route: while Bevacizumab is administered intravenously and has more potential for systemic complications; Ranibizumab is administered in the eye and the systemic exposure is reduced.

Other significant difference between the two anti-VEGF mAbs are the reports of *Eye disorders* & *Infections and Infestations*. Once again, these can be explained by the differences of the mAbs. While Bevacizumab systemic distribution does seem to have major impact at eye-level nor on arising infections, Ranibizumab is administered directly in the Eye – administration in such site can not only potentiate *Eye disorders* but can also enhance the possibility of *Infections and Infestations* at eye-level.

Although many discrepancies were observed in the two mAbs of anti-VEGF class, these differences were highly related to the characteristics of each mAb and do not contradict the concept of main AEs reported being highly correlated to mAbs mechanism of action. Nevertheless, it was interesting to see that there some key factors that cannot be excluded when analysing the safety profiles of mAbs – amdnistration routes, mAbs structural configurations and profile of patients receiving those mAbs may have a significant impact and this was observed for anti-VEGF mAbs.

I. Anti-CD20 mAbs

Many therapeutic strategies have been explored that use mAb or their derivatives in the treatment of cancer, and some promising therapeutic possibilities have already emerged. Rituximab (Mabthera) is the first mAb approved for the treatment of cancer [32].

Antibodies to CD20 have confirmed the hypothesis that monoclonal reagents can be given in vivo to alleviate human diseases. The targeting of CD20 on normal, malignant and auto-immune B-lymphocytes by rituximab has demonstrated substantial benefits for patients with a variety of B-cell lymphomas, as well as some with autoimmune disorders. There has been a notable increase in the survival rates from B-cell lymphoma in the decade since anti-CD20 therapy was introduced.

CD20 mAbs can be classified into type I and type II based on their ability to induce the reorganization of CD20 molecules into lipid rafts upon binding. Type I CD20 mAbs induce the reorganization of CD20 molecules into lipid rafts and efficiently activate the classical pathway of the complement system. In contrast, type II CD20 mAbs poorly activate complement, but are capable to directly induce cell death upon binding to CD20 without cross-linking by secondary Abs. Both types are capable of inducing antibody dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells [110].

Anti-CD20 mAbs appear to eliminate their targets by engaging in a range of effector pathways. These include mAb Fc-FcγR interactions, including antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis, complement-dependent cytotoxicity (CDC), and the direct induction of programmed cell death (PCD).14 More recently, evidence has emerged to suggest a potential role for passive antibody induced immunization [111, 112].

Rituximab was originally shown to be capable of binding C1q and inducing complement-mediated cell lysis. Subsequent work confirmed this and it is clear that CD20 is an excellent target for Complement-Dependent Toxicity (CDC) against numerous cell types in vitro probably, at least in part, because of its high expression and the proximity of the mAb-binding-epitope to the plasma membrane.

Nevertheless, it should be noted that C1q has numerous other effects in vivo including a critical role in the phagocytosis of apoptotic bodies and effects on APC maturation and function. Also, recent data suggests that complement is not an important component for clearance of tumour and may indeed even be detrimental to therapeutic efficacy.

There is no doubt that Rituximab does effectively engage C1q and induces CDC in lymphoma cell lines and primary tumour cells in vitro because of its ability to rapidly redistribute CD20 into membrane lipid rafts. The importance of this mechanism in the elimination of B cells in vivo does, however, remain controversial [111, 112].

It has also been proposed that mAb binding of CD20 can directly transmit intracellular signals that lead to Programmed Cell Death (PCD). However it has never been formally shown what molecular process in vivo might mimic the high affinity cross linking achieved with mAb reagents in vitro, although it is postulated that this could be performed by FcgR-bearing effector cells. As for CDC, the support for rituximab promoting cell death is apparent, but whether this mechanism is critical for the depletion of CD20 positive target cells in vivo remains to be proven [111].

Although the evidence regarding the involvement of CDC and PCD remains inconclusive, it is clear that Fc:FcgR interactions are perhaps the most critical for the efficacy of anti- CD20 immunotherapy. FcgR are expressed on immune cells such as monocytes, macrophages, natural killer cells and neutrophils. FcgR-dependent activation of these immune effectors potentially leads to the release of inflammatory mediators and/or killing/direct phagocytosis of the opsonised target cells. However, the outcome of these mAb: effector cell interactions varies markedly, dependent on both the cell type and balance of activatory and inhibitory FcgR signalling induced.

In syngeneic mouse model systems, using either mouse anti-mouse CD20 mAb in wild-type mice or anti-human CD20 mAb in human CD20 transgenic mice, a complete absence of normal B-cell depletion has been observed in mice lacking the common g chain, indicating an absolute requirement in vivo for activatory FcgR interactions. However, it still remains to be determined which of the FcgR-expressing immune effector cells are critical [111, 112].

Mechanisms such as CDC, ADCC and PCD are considered to be immediate and comparatively short-acting, but the clinical response to a single course of mAb can be late acting and prolonged. This has led to the suggestion that anti-CD20 mAb could also have an immunization effect. Rituximab-induced cell death, by the three main pathways described, will result in release of tumour antigens and changes in localized inflammation. Such an environment promotes the uptake of tumour-associated antigens by dendritic cells and cross-presentation to T lymphocytes, providing the potential for cell mediated immunity. It is yet to be concluded whether this immunization effect correlates with clinical outcome [111].

As detailed earlier, the main success of anti-CD20 mAb has been in combination with chemo- or radiotherapy. Addition of Rituximab to standard front-line chemotherapy regimens significantly improves ORR, CR and OS in low-grade non-Hodgkin's lymphoma and newly diagnosed patients with diffuse large B-cell lymphoma. The mechanism of this synergistic activity is not clear [111].

1. mAbs targeted for CD20

Table 22 – anti-CD20 mAbs approved indications for Europe and for the United States

Anti-CD20 mAbs						
RITUXIMAB		**OFATUMUMAB**		**IBRITUMOMAB TIUXETAN**		**Indications**
U.S.	**EU**	**U.S.**	**EU**	**U.S.**	**EU**	
X	X			X	X	*Follicular Lymphoma NHL*
X	X					*Diffuse Large B cell NHL*
X	X	X	X			*Chronic lymphocytic leukaemia (CLL)*
X	X					*Rheumatoid arthritis*
X	X					*Granulomatosis with polyangiitis and microscopic polyangiitis*

1.1. RITUXIMAB

Rituximab (trade name MabThera/RITUXAN in Europe and United States, respectively) is currently in the market with the following indications:

- Two forms of **non−Hodgkin's Lymphoma**
  - follicular lymphoma
  - diffuse large B cell non-Hodgkin's lymphoma
- **Chronic Lymphocytic Leukaemia (CLL)**
- **Rheumatoid Arthritis (RA)**
- Two forms of severe vasculitis
  - **Granulomatosis with Polyangiitis** (GPA) (also called, Wegener's granulomatosis)
  - **Microscopic Polyangiitis** (MPA)

Rituximab, discovered by Biogen Idec, first received FDA approval for the treatment of relapsed indolent non-Hodgkin Lymphoma (NHL) in 1997 and was the first targeted cancer medicine approved by the U.S. Food and Drug Administration [1]. Rituximab was approved under the trade name MabThera in the EU in June 1998.

Rituximab is known as RITUXAN in the United States, Japan and Canada.

Genentech, a member of the Roche Group, and Biogen Idec collaborate on Rituximab in the United States. Roche markets Rituximab in the rest of the world, except Japan, where Rituximab is co-marketed by Chugai and Zenyaku Kogyo Co. Ltd [113].

1.1.1. RITUXIMAB Characterization

Pharmacotherapeutic group: Antineoplastic agents
ATC code: L01XC02 [57]

L ANTINEOPLASTIC AND IMMUNOMODULATING AGENTS
L01 ANTINEOPLASTIC AGENTS
L01X OTHER ANTINEOPLASTIC AGENTS
L01XC Monoclonal antibodies

Rituximab is a genetically engineered chimeric murine/human monoclonal antibody representing a glycosylated immunoglobulin with human IgG1 constant regions (Fc domain) and murine light-chain and heavy chain variable region sequences (Fab domain).

Rituximab is directed against the CD20 antigen and it has a binding affinity for the CD20 antigen of approximately 8.0 nM.

Rituximab has an approximate molecular weight of 145 kD. The antibody is produced by mammalian (Chinese hamster ovary) cell suspension culture and purified by affinity chromatography and ion exchange, including specific viral inactivation and removal procedures [114, 115].

1.1.2. RITUXIMAB Mechanism of Action

Rituximab is a monoclonal antibody that targets the CD20, a non-glycosylated phosphoprotein, located on pre-B and mature B-lymphocytes. The antigen is expressed on >95 % of all B cell non-Hodgkin's lymphomas.

CD20 is found on both normal and malignant B cells, but not on haematopoietic stem cells, pro-B cells, normal plasma cells or other normal tissue. The CD20 antigen has characteristics that render it a suitable target for treatment:

- CD20 does not circulate freely in the plasma,
- CD20 does not shed from the surface of B-cells after binding of anti-CD20 antibodies,
- CD20 does not internalise upon antibody binding.

The potential therapeutic advantage of chimeric antibodies as compared with pure murine mAbs is the reduction of immunogenicity thereby permitting repeated administration. Other important features of chimeric mAbs are their ability of binding human complement (C1q), and the mediation of human effector functions such as complement fixation and antibody-dependent cellular cytotoxicity resulting in a potentially more effective destruction of tumour cells.

The Fab domain of rituximab binds to the CD20 antigen on B lymphocytes and the Fc domain can recruit immune effector functions to mediate B cell lysis. Possible mechanisms of cell lysis include complement dependent cytotoxicity (CDC) resulting from C1q binding, and antibody-dependent cellular cytotoxicity (ADCC) mediated by one or more of the Fcγ receptors on the

surface of granulocytes, macrophages and NK cells. Rituximab binding to CD 20 antigen on B lymphocytes has also been demonstrated to induce cell death via apoptosis.

B cells are believed to play a role in the pathogenesis of rheumatoid arthritis (RA) and associated chronic synovitis. In this setting, B cells may be acting at multiple sites in the autoimmune/inflammatory process, including through production of rheumatoid factor (RF) and other autoantibodies, antigen presentation, T-cell activation, and/or pro-inflammatory cytokine production [114-116].

1.1.3. RITUXIMAB Treatment

Table 23 – Rituximab Treatment: Method of administration and Indications

RITUXIMAB | *Administration:* intravenous infusion					
U.S.	**EU**	**Indications**	**Population**	**Indications Details**	**References**
X	X	***Follicular Lymphoma NHL***	Adults	– in combination with chemotherapy for previously untreated patients with stage III-IV follicular lymphoma – maintenance therapy for follicular lymphoma patients responding to induction therapy – monotherapy for stage III-IV follicular lymphoma who are chemoresistant or are in their second or subsequent relapse after chemotherapy	[114, 115]
X	X	***Diffuse Large B cell NHL***	Adults	– in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) chemotherapy for CD20 positive diffuse large B cell NHL	[114, 115]
X	X	***Chronic lymphocytic leukaemia (CLL)***	Adults	– in combination with chemotherapy for patients with previously untreated and relapsed/refractory chronic lymphocytic leukaemia	[114, 115]
X	X	***Rheumatoid arthritis***	Adults	– in combination with methotrexate for severeactive rheumatoid arthritis which has had an inadequate response or intolerance to other diseasemodifying anti-rheumatic drugs (DMARD), including one or more tumour necrosis factor (TNF) inhibitor therapies.	[114, 115]
X	X	***Granulomatosis with polyangiitis and microscopic polyangiitis***	Adults	– in combination with glucocorticoids for the induction of remission of severe, active granulomatosis with polyangiitis (Wegener's) (GPA) and microscopic polyangiitis (MPA).	[114, 115]

1.1.1. RITUXIMAB Adverse Events (as per EU Initial MA documents)

Table 24 – Rituximab Adverse Events as per European Initial Marketing-authorisation Documents

MedDRA 17.1 System Organ Class (SOC)	AEs Detailed	References
General disorders and administration site conditions	– Chills – Fever	[116]
Immune system disorders	– Cytokine release syndrome	[116]
Infections and Infestations	– Bacterial infections – Viral infection	[116]
Metabolism and nutrition disorders	– Rapid tumour lysis and features of tumour lysis syndrome	[116]
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	– Paraneoplastic pemphigus	[116]
Respiratory, thoracic and mediastinal disorders	– Bronchospasm – Dyspnoea – Hypoxaemia (in those patients who had oxygen saturation and/or oxygen tension assessed) – Pulmonary infiltrates (in those patients who had chest x-ray performed)	[116]
Skin and subcutaneous tissue disorders	– Angioedema – Lichen planus – Lichenoid dermatitis – Toxic epidermal necrolysis	[116]
Vascular disorders	– Flushing – Hypotension	[116]

1.1.1. RITUXIMAB Adverse Events Charts (following MA)

- ***European Data (EU database of suspected adverse drug reactions reports)***

The data here displayed was launched by EMA in the European database of suspected adverse drug reactions reports. Reports included in this database correspond to the data submitted electronically to EudraVigilance by national medicines regulatory authorities and by pharmaceutical companies that hold marketing authorisations (licences) for the medicines.

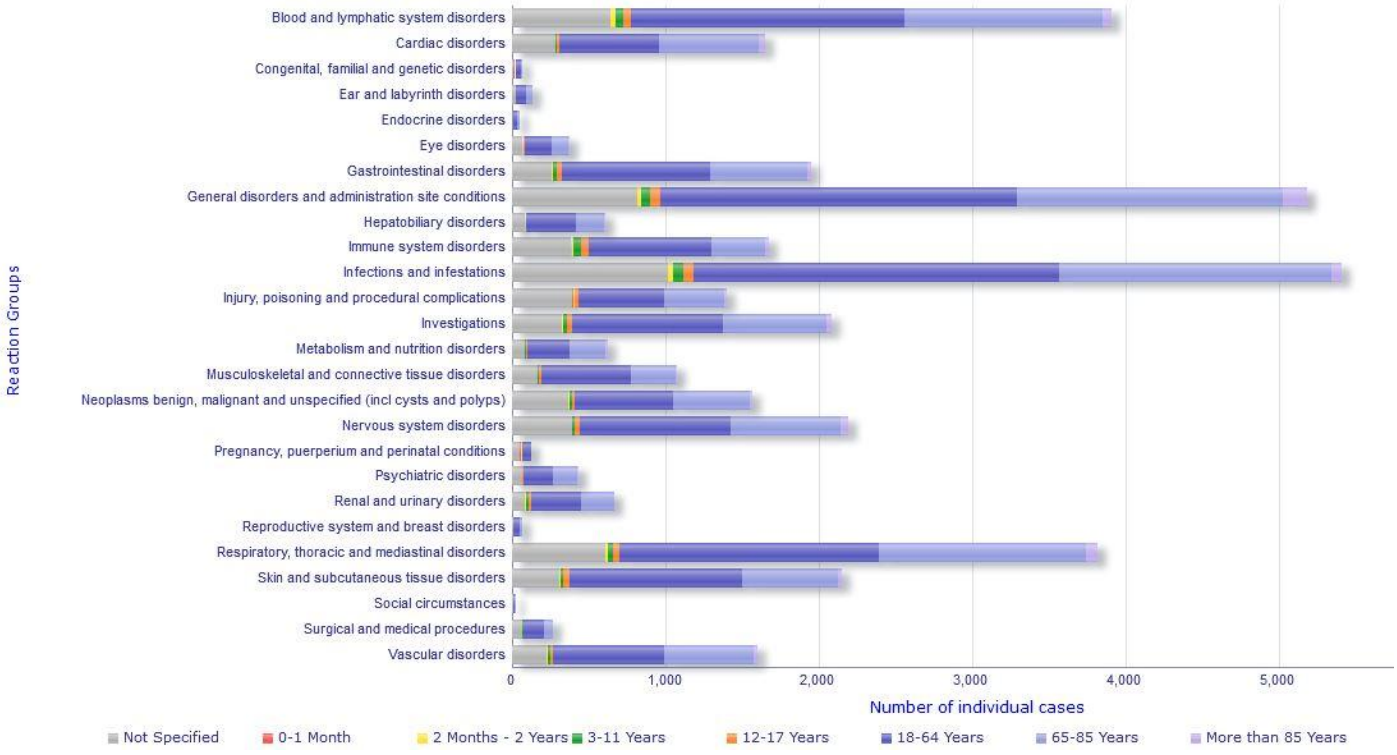

Chart 19 – Individual cases sorted by reactions groups, submitted for RITUXIMAB (up to Oct'14) *in* European database of suspected adverse drug reaction reports (www.adrreports.eu) (data extracted on 25-Nov-2014)

The chart above displays the number of cases reported for each reaction group, divided by age. The original chart (online format) enabled to pass the mouse through each bar and reveal the number of cases for a specific age. Below are the values of the biggest bars.

EU Top 10 of Individual Cases (by reactions groups):

1. Infections and Infestations (5406 cases)
2. General disorders and administration site conditions (5183 cases)
3. Blood ans lymphatic system disorders (3909 cases)
4. Respiratory, thoracic and mediastinal disorders (3817 cases)
5. Nervous system disorders (2190 cases)
6. Skin and subcutaneous tissue disorders (2150 cases)
7. Investigations (e.g. Chest X-ray abnormal; Electocardiogram QT prolonged) (2084 cases)
8. Gastrointestinal disorders (1954 cases)
9. Immune system disorders (1672 cases)
10. Cardiac disorders (1648 cases)

- ***FDA Adverse Event Reporting System (FAERS) Data (DrugCite.com)***

The data here displayed was made available by DrugCite database. Results included in this database correspond to the collection and analysis of reported adverse performed by FDA Adverse Events Reporting System (FAERS). These reports are submitted by physicians, healthcare consumers, lawyers amongst others.

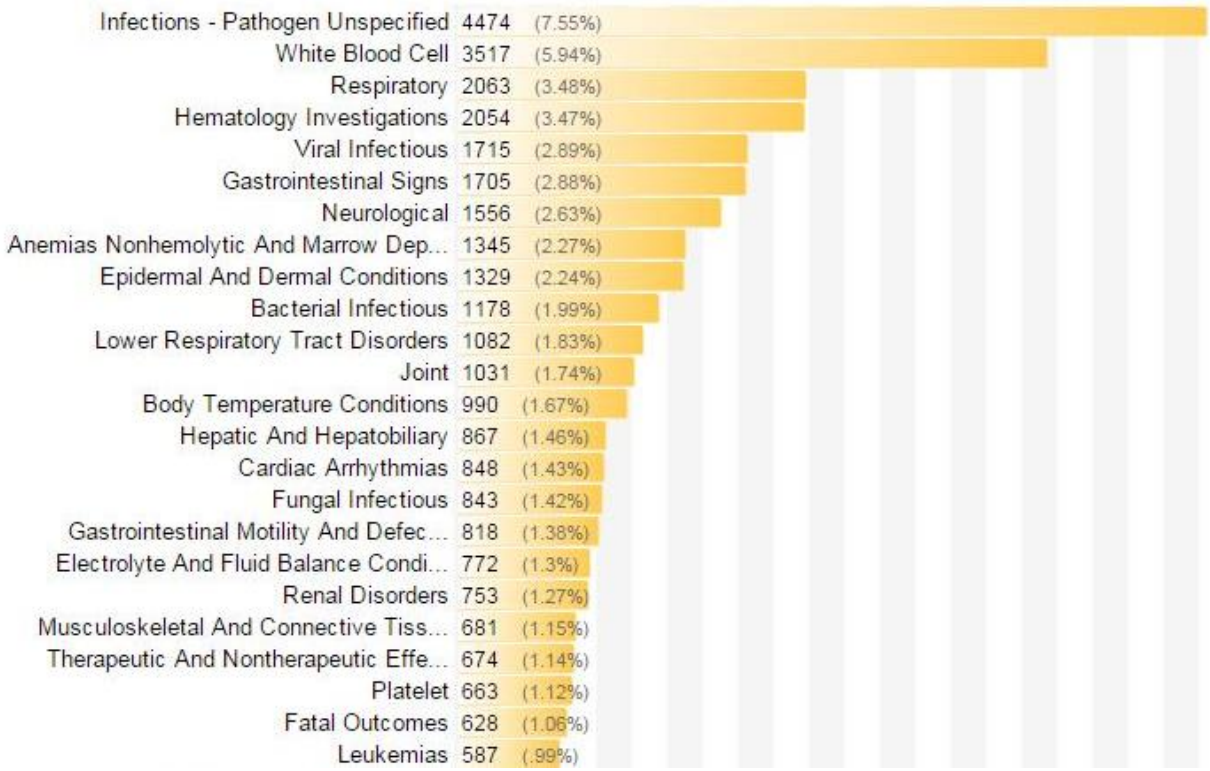

Chart 20 – Individual cases sorted by reactions groups, submitted for RITUXIMAB and other drugs with same active substance (Q1 2004 – Q3 2012) *in* DrugCite.com (www.drugcite.com)

(data extracted on 25-Nov-2014)

The chart above displays, for each reaction group, the number of cases reported where the drug was flagged as a suspect drug causing the adverse event. All percentages (%) are the cases reported for a specific reaction group within the sum up of all reported cases in all reactions groups (percent does not represent rate related to drug utilization).

U.S. Top 10 of Individual Cases (by reactions groups):

1. Infections – Pathogen Unspecified (4474 cases)
2. White Blood Cell (e.g. Febril Neutropenia; Leukopenia) (3517 cases)
3. Respiratory (e.g. Cough; Hypoxia; Lung Disorder) (2063 cases)
4. Hematology Investigations (e.g. Haemoglobin/White Blood Cell Count) (2054 cases)
5. Viral Infections (1715 cases)
6. Gastrointestinal Signs (1705 cases)
7. Neurological (e.g. Syncope; Dizziness; Somnolence) (1556 cases)
8. Anemias Nonhemolytic And Marrow Depression (1345 cases)
9. Epidermal And Dermal Conditions (1329 cases)
10. Bacterial Infections (1178 cases)

- ***Comparison of Adverse Events reported in EU and in the U.S.***

Following the analysis of the Adverse Events previously presented, it became clear that there were some differences between EU data and U.S. data. In order to have a visually enhanced perception of such differences, it was decided to create a cumulative chart which would aggregate all the data previously presented:

- Post Marketing EU data (Blue bars in the cumulative chart)
- Post Marketing U.S. data (Orange bars in the cumulative chart)
- EU data as per Initial documents for MA (MedDRA SOCs highlighted in yellow in the cumulative chart)

For aggregation purposes, a common classification had to be used and so MedDRA SOC was the selected classification for the cumulative chart below presented.

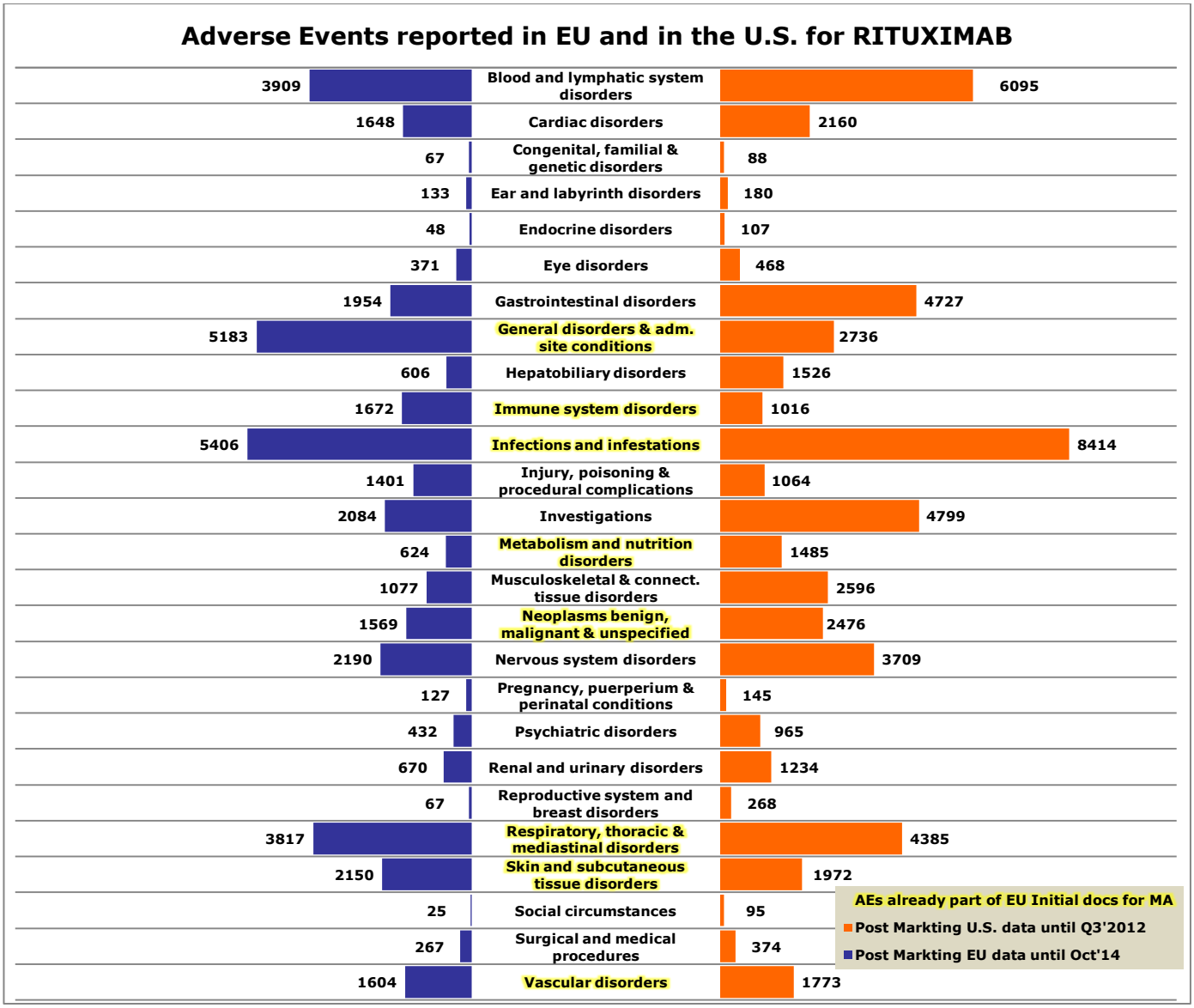

Chart 21 – Individual cases sorted by reactions groups (MedDRA 17.1 SOC), submitted for RITUXIMAB prior and post MA in Europe and post MA the United States

Comments to RITUXIMAB cumulative chart:

1. SOCs included in EU pre-MA reports vs SOCs included in EU post-MA reports

Main discrepancies: There are cases of SOCs with a considerable number of AE reports (>1600) in post-MA and which were not foreseen by pre-MA clinical trials data. These include*: Blood and lymphatic system disorders*; *Cardiac disorders*; *Gastrointestinal disorders*; *Investigations* & *Nervous system disorders*.

2. SOCs included in EU post-MA reports vs. SOCs included in U.S. post-MA reports

Although Rituximab is approved in EU and in the U.S. for the same indications, there are some significant discrepancies in the numbers above observed.

Main discrepancies: Having EU data as reference, there were some U.S. bars which were expected to be bigger but are not. These include: *General disorders and administration site conditions*; *Immune system disorder*; *Injury, poisoning and procedural complications* & *Neoplasms benign, malignant and unspecified (including cysts and polyps)*.

1.2. OFATUMUMAB

Europe and the United States share the approval of Ofatumumab (trade name ARZERRA) for the treatment of chronic lymphocytic leukaemia (CLL), a cancer of a type of white blood cells called lymphocytes.

Ofatumumab is manufactured by Glaxo Group Ltd and has been approved in the U.S and Europe since 2009 and 2010, respectively [117, 118].

Ofatumumab is designated as an orphan medicinal product in the EU for the indication: "treatment of chronic lymphocytic leukaemia" (EU/3/08/581). The Committee for Orphan Medicinal Products (COMP) concluded that chronic lymphocytic leukaemia was estimated to be affecting approximately 3.5 in 10,000 persons in the Community, at the time the application was made (June 2008). This is equivalent to a total of around 176,000 people, and is below the threshold for orphan designation, which is 5 people in 10,000. Also, Chronic Lymphocytic Leukaemia is a condition chronically debilitating and life-threatening, in particular due to poor long-term survival in high-risk patients [119, 120].

***Criteria for Orphan designation (as per Regulation (EC) No 141/2000, article 3)**:*

A medicinal product shall be designated as an orphan medicinal product if its sponsor can establish:

I. that it is intended for the diagnosis, prevention or treatment of a life-threatening or chronically debilitating condition affecting not more than five in 10 thousand persons in the Community when the application is made,

or

that it is intended for the diagnosis, prevention or treatment of a life-threatening, seriously debilitating or serious and chronic condition in the Community and that without incentives it is unlikely that the marketing of the medicinal product in the Community would generate sufficient return to justify the necessary investment;

and

II. that there exists no satisfactory method of diagnosis, prevention or treatment of the condition in question that has been authorised in the Community or, if such method exists, that the medicinal product will be of significant benefit to those affected by that condition [121].

Sponsors who obtain orphan designation benefit from a number of incentives, including protocol assistance, a type of scientific advice specific for designated orphan medicines, and market exclusivity once the medicine is on the market. Fee reductions are also available depending on the status of the sponsor and the type of service required [121, 122].

1.2.1. OFATUMUMAB Characterization

Pharmacotherapeutic group: Monoclonal antibodies
ATC code: L01XC10 [57]

L ANTINEOPLASTIC AND IMMUNOMODULATING AGENTS
L01 ANTINEOPLASTIC AGENTS
L01X OTHER ANTINEOPLASTIC AGENTS
L01XC Monoclonal antibodies

Ofatumumab is an IgG1κ human monoclonal antibody with a molecular weight of approximately 149 kDa. The antibody was generated via transgenic mouse and hybridoma technology and is produced in a recombinant murine cell line (NS0) using standard mammalian cell cultivation and purification technologies [117].

1.2.2. OFATUMUMAB Mechanism of Action

Ofatumumab is a human monoclonal antibody (IgG1) that binds specifically to a distinct epitope encompassing both the small and large extracellular loops of the CD20 molecule.

The CD20 molecule is a transmembrane phosphoprotein expressed on B lymphocytes from the pre-B to mature B lymphocyte stage and on B cell tumours. The B cell tumours include CLL (generally associated with lower levels of CD20 expression) and non-Hodgkin's lymphomas (where > 90% tumours have high levels of CD20 expression). The CD20 molecule is not shed from the cell surface and is not internalised following antibody binding.

Ofatumumab induces cross linking of CD20 molecules and relocation of these CD20 molecules to so-called lipid rafts considered important for induction of cell signalling and effective complement activation. The binding of Ofatumumab to the membrane-proximal epitope of the CD20 molecule induces recruitment and activation of the complement pathway at the cell surface, leading to complement-dependent cytotoxicity (CDC) and resultant lysis of tumour cells.

Ofatumumab has been shown to induce appreciable lysis of cells with high expression levels of complement defence molecules. Ofatumumab has also been shown to induce cell lysis in both high and low CD20 expressing cells and in rituximab-resistant cells. In addition, the binding of Ofatumumab allows the recruitment of natural killer cells allowing the induction of cell death through antibody-dependent cell-mediated cytotoxicity (ADCC). The resulting depletion of malignant B cells carrying the CD20 epitope by Ofatumumab treatment is considered the main mechanism of action leading to clinical benefit to subjects with CD20-expressing cell tumours. [118, 119].

1.2.3. OFATUMUMAB Treatment

Table 25 – Ofatumumab Treatment: Method of administration and Indications

OFATUMUMAB | *Administration:* intravenous infusion					
U.S.	**EU**	**Indications**	**Population**	**Indications Details**	**References**
X	X	***Previously untreated Chronic Lymphocytic Leukaemia (CLL)***	Adults	– in combination with chlorambucil or bendamustine for patients with CLL who have not received prior therapy and who are not eligible for fludarabine-based therapy	[117, 118]
X	X	***Refractory CLL***	Adults	– treatment of CLL in patients who are refractory to fludarabine and alemtuzumab	[117, 118]

1.1.1. OFATUMUMAB Adverse Events (as per EU Initial MA documents)

Table 26 – Ofatumumab Adverse Events as per European Initial Marketing-authorisation Documents

MedDRA 17.1 System Organ Class (SOC)	AEs Detailed	References
Blood and lymphatic system disorders	– Anaemia – Febrile neutropenia – Leukopenia – Neutropenia – thrombocytopenia	[119]
Cardiac disorders	– Tachycardia	[119]
Gastrointestinal disorders	– Diarrhoea – Nausea – Small bowel obstruction	[119]
General disorders and administration site conditions	– Chest discomfort – Chills – Fatigue – Pyrexia – Rigors	[119]
Immune system disorders	– Anaphylactoid reactions – Cytokine release syndrome – Hypersensitivity	[119]
Infections and Infestations	– Herpes virus infection – Lower respiratory tract infection, including pneumonia – Sepsis, including neutropenic sepsis and septic shock – Upper respiratory tract infection – Urinary tract infection	[119]
Musculoskeletal and connective tissue disorders	– Back pain	[119]
Respiratory, thoracic and mediastinal disorders	– Bronchospasm – Cough – Dyspnoea – Hypoxia – Nasal congestion – Pharyngolaryngeal pain	[119]
Skin and subcutaneous tissue disorders	– Hyperhidrosis – Pruritus – Rash – Urticaria	[119]
Vascular disorders	– Flushing – Hypertension – Hypotension	[119]

1.1.1. OFATUMUMAB Adverse Events Charts (following MA)

- ***European Data (EU database of suspected adverse drug reactions reports)***

The data here displayed was launched by EMA in the European database of suspected adverse drug reactions reports. Reports included in this database correspond to the data submitted electronically to EudraVigilance by national medicines regulatory authorities and by pharmaceutical companies that hold marketing authorisations (licences) for the medicines.

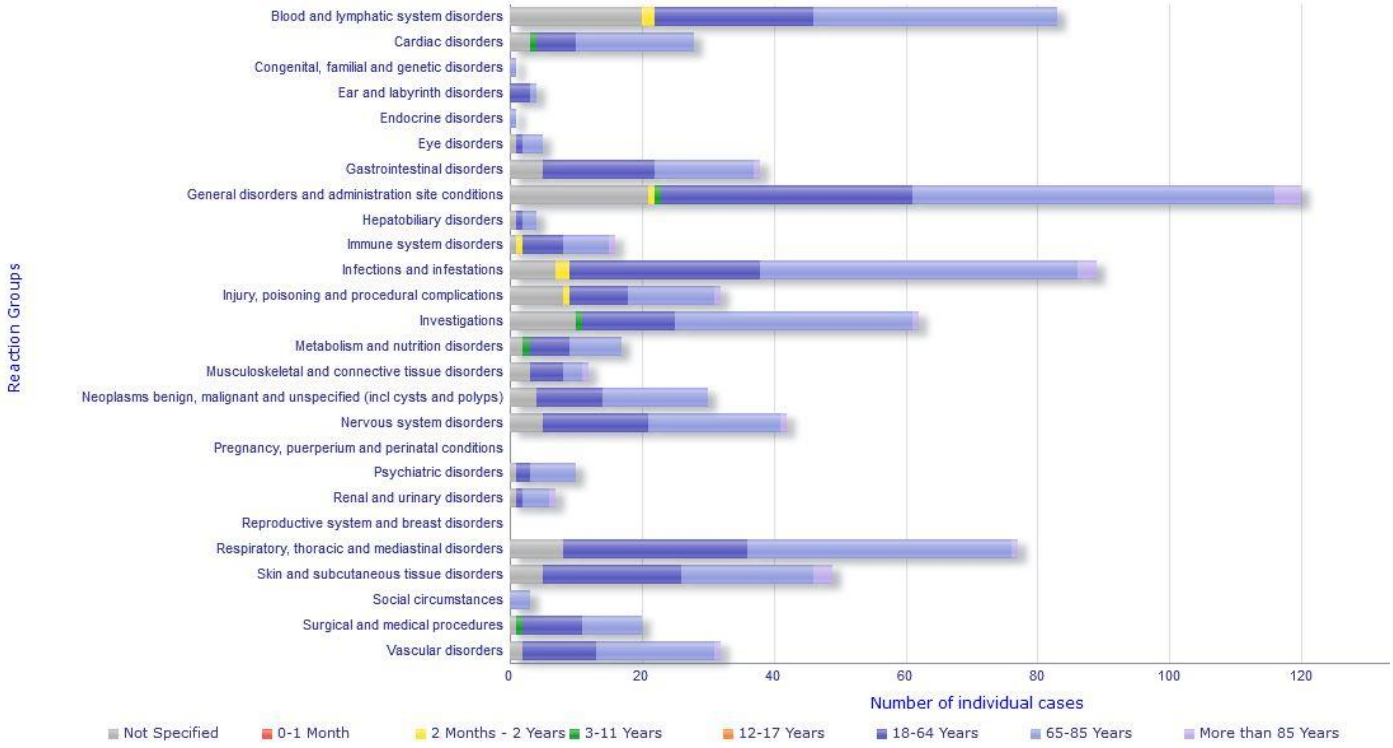

Chart 22 – Individual cases sorted by reactions groups, submitted for OFATUMUMAB (up to Oct'14)
***in* European database of suspected adverse drug reaction reports (www.adrreports.eu)**
(data extracted on 25-Nov-2014)

The chart above displays the number of cases reported for each reaction group, divided by age. The original chart (online format) enabled to pass the mouse through each bar and reveal the number of cases for a specific age. Below are the values of the biggest bars.

EU Top 10 of Individual Cases (by reactions groups):

1. General disorders and administration site conditions (120 cases)
2. Infections and Infestations (89 cases)
3. Blood ans lymphatic system disorders (83 cases)
4. Respiratory, thoracic and mediastinal disorders (77 cases)
5. Investigations (e.g. Chest X-ray abnormal; Electocardiogram QT prolonged) (62 cases)
6. Skin and subcutaneous tissue disorders (49 cases)
7. Nervous system disorders (42 cases)
8. Gastrointestinal disorders (38 cases)
9. Vascular disorders (32 cases)
10. Injury, poisoning and procedual complications (32 cases)

- ***FDA Adverse Event Reporting System (FAERS) Data (DrugCite.com)***

The data here displayed was made available by DrugCite database. Results included in this database correspond to the collection and analysis of reported adverse performed by FDA Adverse Events Reporting System (FAERS). These reports are submitted by physicians, healthcare consumers, lawyers amongst others.

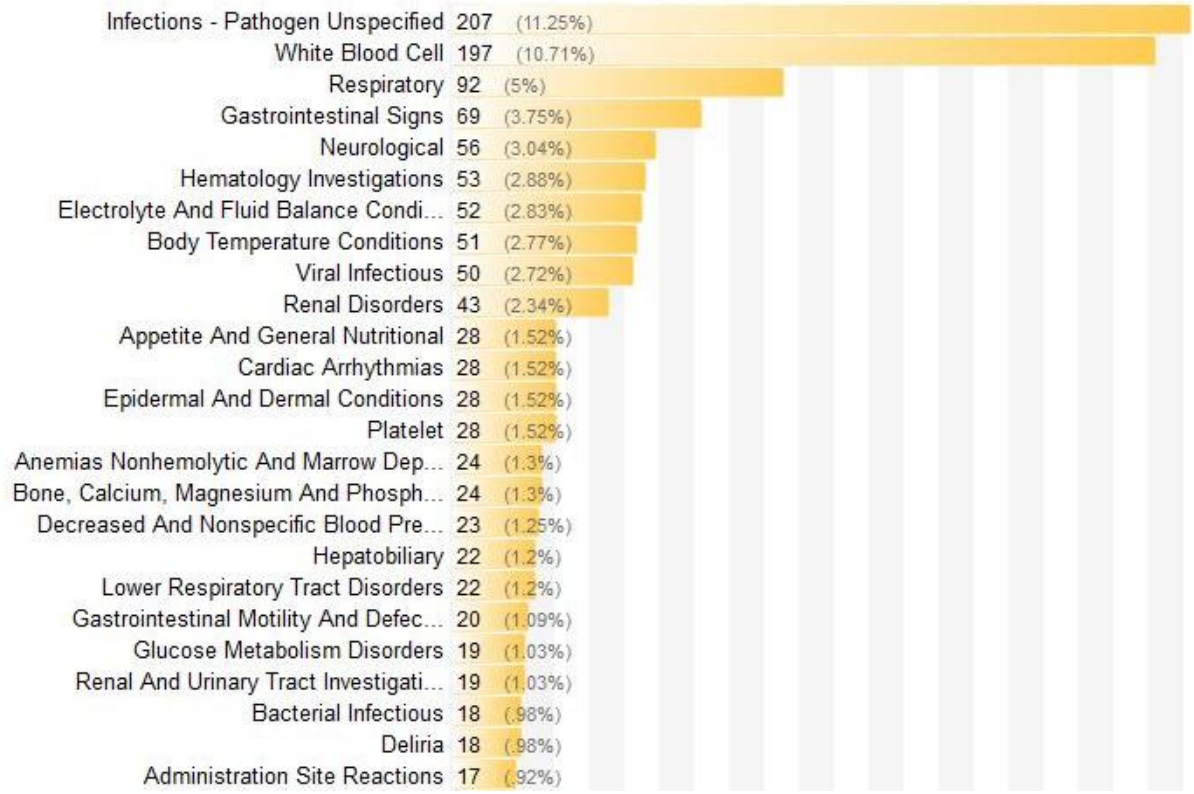

Chart 23 – Individual cases sorted by reactions groups, submitted for OFATUMUMAB and other drugs with same active substance (Q1 2004 – Q3 2012) *in* DrugCite.com (www.drugcite.com)

(data extracted on 25-Nov-2014)

The chart above displays, for each reaction group, the number of cases reported where the drug was flagged as a suspect drug causing the adverse event. All percentages (%) are the cases reported for a specific reaction group within the sum up of all reported cases in all reactions groups (percent does not represent rate related to drug utilization).

U.S. Top 10 of Individual Cases (by reactions groups):

1. Infections – Pathogen Unspecified (207 cases)
2. White Blood Cell (e.g. Febril Neutropenia; Leukopenia) (197 cases)
3. Respiratory (e.g. Cough; Hypoxia; Lung Disorder) (92 cases)
4. Gastrointestinal Signs (69 cases)
5. Neurological (e.g. Syncope; Dizziness; Somnolence) (56 cases)
6. Hematology Investigations (e.g. Haemoglobin/White Blood Cell Count) (53 cases)
7. Electrolyte And Fluid Balance Conditions (52 cases)
8. Body Temperature Conditions (51 cases)
9. Viral Infections (50 cases)
10. Renal Disorders (43 cases)

- ***Comparison of Adverse Events reported in EU and in the U.S.***

Following the analysis of the Adverse Events previously presented, it became clear that there were some differences between EU data and U.S. data. In order to have a visually enhanced perception of such differences, it was decided to create a cumulative chart which would aggregate all the data previously presented:

- Post Marketing EU data (Blue bars in the cumulative chart)
- Post Marketing U.S. data (Orange bars in the cumulative chart)
- EU data as per Initial documents for MA (MedDRA SOCs highlighted in yellow in the cumulative chart)

For aggregation purposes, a common classification had to be used and so MedDRA SOC was the selected classification for the cumulative chart below presented.

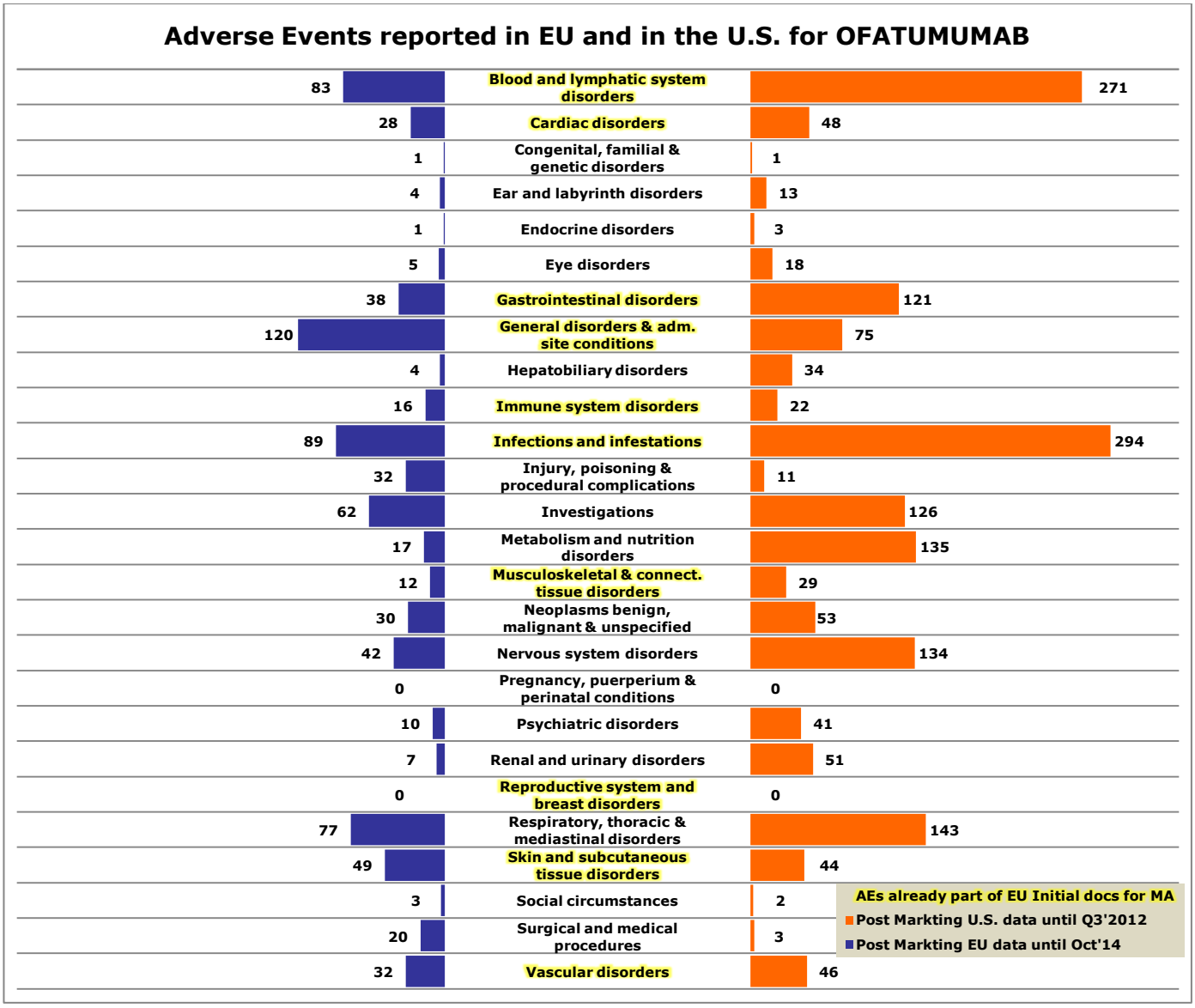

Chart 24 – Individual cases sorted by reactions groups (MedDRA 17.1 SOC), submitted for OFATUMUMAB prior and post MA in Europe and post MA the United States

Comments to OFATUMUMAB cumulative chart:

1. SOCs included in EU pre-MA reports vs SOCs included in EU post-MA reports

Main discrepancies: There are cases of SOCs with a considerable number of AE reports (>30) in post-MA and which were not foreseen by pre-MA clinical trials data. These include*: Injury, poisoning and procedural complications*; *Investigations*; *Neoplasms benign, malignant and unspecified (including cysts and polyps)*; *Nervous system disorders* & *Respiratory, thoracic and mediastinal disorders*.

2. SOCs included in EU post-MA reports vs. SOCs included in U.S. post-MA reports

Although Ofatumumab is approved in EU and in the U.S. for the same indications, there are some significant discrepancies in the numbers above observed.

Main discrepancies: Having EU data as reference, there were some U.S. bars which were expected to be bigger but are not. These include: *General disorders and administration site conditions*; *Injury, poisoning and procedural complications;* & *Psychiatric disorders.*

Based on EU data, there were also some U.S. bars which were expected to be smaller: *Hepatobiliary disorders* & *Metabolism and nutrition disorders*.

1.2. IBRITUMOMAB TIUXETAN

Ibritumomab Tiuxetan (trade name ZEVALIN) is currently in the market with slightly different indications for Europe and for the United Sates.

While in Europe Ibritumomab Tiuxetan is used in the following groups of patients:

- patients who have gone into remission (reduction in the number of cancerous cells) after their first 'induction treatment' (initial chemotherapy treatment) for lymphoma. Zevalin is given as 'consolidation therapy' to improve the remission;
- patients in whom Rituximab (another treatment for non-Hodgkin's lymphoma) is no longer effective or whose disease has come back after rituximab treatment.

In the United States, Ibritumomab Tiuxetan is used for:

- relapsed or refractory, low-grade or follicular B-cell non-Hodgkin's lymphoma (NHL)
- previously untreated follicular NHL who achieve a partial or complete response to first-line chemotherapy

Zevalin is marketed by Spectrum Pharmaceuticals in the United States and by Bayer Schering Pharma in Europe.

1.2.1. IBRITUMOMAB TIUXETAN Characterization

Pharmacotherapeutic group: Therapeutic radiopharmaceuticals
ATC code: V10XX02[57]
V VARIOUS
V10 THERAPEUTIC RADIOPHARMACEUTICALS
V10X OTHER THERAPEUTIC RADIOPHARMACEUTICALS
V10XX Various therapeutic radiopharmaceuticals

Zevalin contains ibritumomab, an IgG1 kappa immunoglobulin produced in Chinese hamster ovary (CHO) cells which reacts specifically with the CD20 antigen found on the surface of normal and malignant B lymphocytes (inc. mature B cells, activated proliferating B cells and differentiating B cells), targets of its cytotoxicity.

Ibritumomab Tiuxetan, IDEC-2B8-MX-DTPA is defined as the active substance in Zevalin. It is obtained by chemically linking the monoclonal antibody ibritumomab to the amino directed bifunctional chelate MX-DTPA (Tiuxetan), via a covalent, urea type bond. This linker-chelator provides a high affinity, conformationally restricted chelation site for Yttrium-90.

The approximate molecular weight of ibritumomab Tiuxetan is 148 kD.

Ibritumomab Tiuxetan achieves selective targeting CD20+ cells, which are inherently sensitive to radiation. The radionucleide yttrium-90 (half-life of 64 hours) emits pure high-energy beta radiation with a local tissue penetration (5 to 10 mm) and effect.

The proposed Zevalin regimen combines the antibody-based tumour cell killing Rituximab with an antibody based radioimmuno-therapy and thus further tumour-cell killing, but with a different mechanism of action (radiation) [123, 124].

1.2.2. IBRITUMOMAB TIUXETAN Mechanism of Action

Ibritumomab Tiuxetan binds specifically to the CD20 antigen. The apparent affinity (KD) of ibritumomab Tiuxetan for the CD20 antigen ranges between approximately 14 to 18 nM.

The CD20 antigen is expressed on pre-B and mature B lymphocytes and on > 90% of B-cell non-Hodgkin's lymphomas (NHL). The CD20 antigen is not shed from the cell surface and does not internalize upon antibody binding.

The chelate Tiuxetan, which tightly binds Y-90, is covalently linked to ibritumomab. The beta emission from Y-90 induces cellular damage by the formation of free radicals in the target and neighboring cells [124].

1.2.3. IBRITUMOMAB TIUXETAN Treatment

Table 27 – Ibritumomab Tiuxetan Treatment: Method of administration and Indications

IBRITUMOMAB TIUXETAN | *Administration:* intravenous infusion (radiopharmaceutical preparation)					
U.S.	**EU**	**Indications**	**Population**	**Indications Details**	**References**
X	X	***Relapsed or refractory follicular B-cell NHL***	Adults	– (EU) patients in whom Rituximab (another treatment for non-Hodgkin's lymphoma) is no longer effective or whose disease has come back after rituximab treatment. – (U.S.) relapsed or refractory, low-grade or follicular B-cell NHL	[123-125]
X	X	***Previously untreated Follicular NHL***	Adults	– (EU) patients who have gone into remission (reduction in the number of cancerous cells) after their first 'induction treatment' (initial chemotherapy treatment) for lymphoma. Ibritumomab Tiuxetan is given as 'consolidation therapy' to improve the remission; – (U.S.) previously untreated follicular NHL who achieve a partial or complete response to first-line chemotherapy	[123-125]

Ibritumomab Tiuxetan is supplied as kit for radiopharmaceutical preparation for intravenous use. It contains four components, including all the non-radioactive ingredients necessary to produce a single dose of [90Y] Ibritumomab Tiuxetan:

- 1 vial of ibritumomab Tiuxetan 1.6 mg/ml
- 1 vial of 50 mM sodium acetate
- 1 vial of formulation buffer
- 1 empty reaction vial

Yttrium-90 [90Y] is not part of the kit and should be provided by the end-user.

The container is the same for all components; a colourless Type I glass vial, teflon-faced gray bromobutyl rubber stopper, alu-cap, different coloured flip-off seal [123, 124].

1.1.1. IBRITUMOMAB TIUXETAN Adverse Events (as per EU Initial MA documents)

Table 28 – Ibritumomab Tiuxetan Adverse Events as per European Initial Marketing-authorisation Documents

MedDRA 17.1 System Organ Class (SOC)	AEs Detailed	References
Blood and lymphatic system disorders	– Anaemia – Neutropenia – Thrombocytopenia	[123]
Gastrointestinal disorders	– Abdominal pain – Diarrhoea – Nausea – Vomiting	[123]
General disorders and administration site conditions	– Chest pain – Chills – Fever – Pain – Peripheral oedema	[123]
Infections and Infestations	– Bacterial infection – Cold syndrome – Fungal infection – Rhinitis – Sepsis – Sinusitis – Urinary tract infection	[123]
Musculoskeletal and connective tissue disorders	– Arthralgia – Asthenia – Back pain – Myalgia	[123]
Nervous system disorders	– Dizziness – Headache	[123]
Psychiatric disorders	– Anorexia	[123]
Respiratory, thoracic and mediastinal disorders	– Bronchospasm – Increased cough – Throat irritation	[123]
Skin and subcutaneous tissue disorders	– Angioedema – Ecchymosis – Pruritus – Rash	[123]
Vascular disorders	– Flushing – Hypotension	[123]

1.1.1. IBRITUMOMAB TIUXETAN Adverse Events Charts (following MA)

- ***European Data (EU database of suspected adverse drug reactions reports)***

The data here displayed was launched by EMA in the European database of suspected adverse drug reactions reports. Reports included in this database correspond to the data submitted electronically to EudraVigilance by national medicines regulatory authorities and by pharmaceutical companies that hold marketing authorisations (licences) for the medicines.

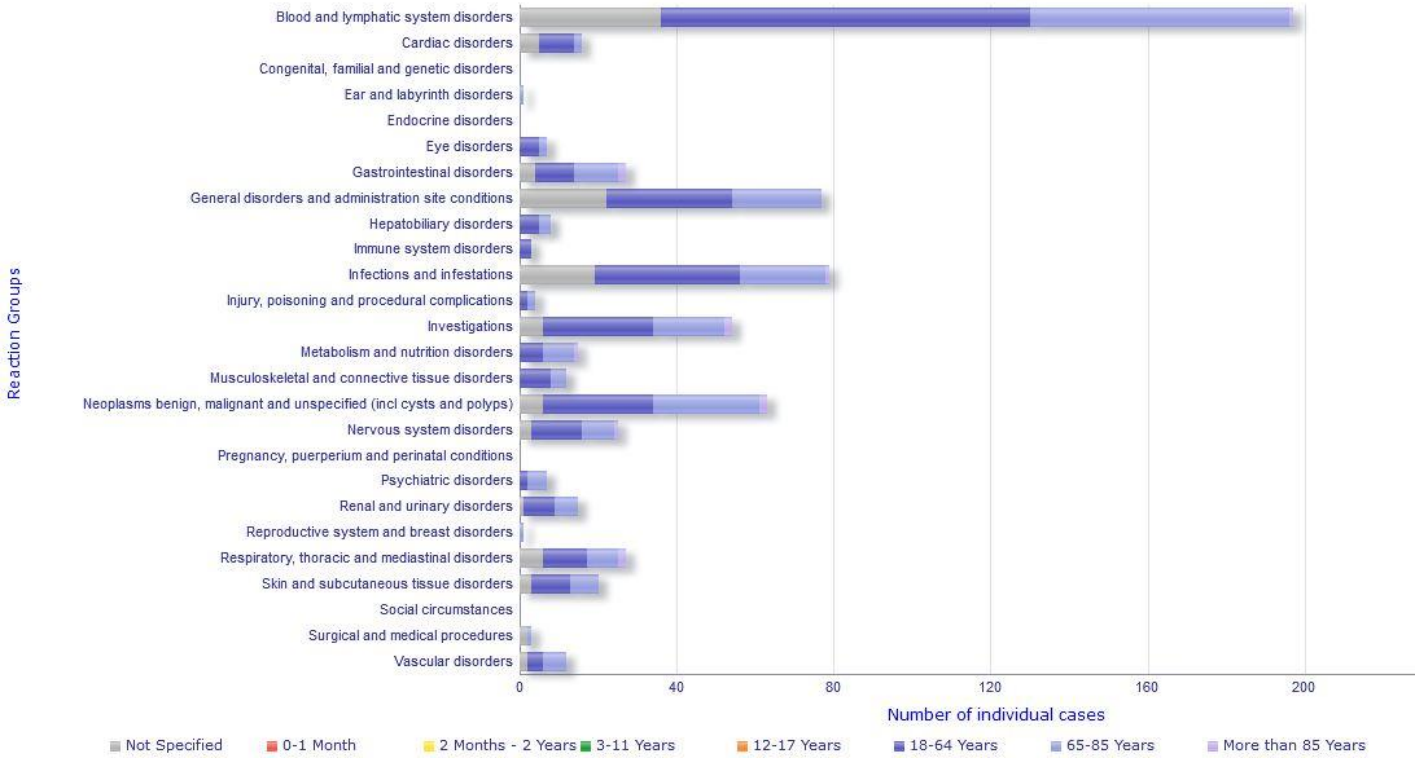

Chart 25 – Individual cases sorted by reactions groups, submitted for IBRITUMOMAB TIUXETAN (up to Oct'14) *in* European database of suspected adverse drug reaction reports (www.adrreports.eu) (data extracted on 25-Nov-2014)

The chart above displays the number of cases reported for each reaction group, divided by age. The original chart (online format) enabled to pass the mouse through each bar and reveal the number of cases for a specific age. Below are the values of the biggest bars.

EU Top 10 of Individual Cases (by reactions groups):

1. Blood ans lymphatic system disorders (197 cases)
2. Infections and Infestations (79 cases)
3. General disorders and administration site conditions (77 cases)
4. Neoplasms benign, malignant and unspecified (including cysts and polyps) (63 cases)
5. Investigations (e.g. Chest X-ray abnormal; Electocardiogram QT prolonged) (54 cases)
6. Respiratory, thoracic and mediastinal disorders (27 cases)
7. Gastrointestinal disorders (27 cases)
8. Nervous system disorders (25 cases)
9. Skin and subcutaneous tissue disorders (20 cases)
10. Cardiac disorders (16 cases)

- ***FDA Adverse Event Reporting System (FAERS) Data (DrugCite.com)***

The data here displayed was made available by DrugCite database. Results included in this database correspond to the collection and analysis of reported adverse performed by FDA Adverse Events Reporting System (FAERS). These reports are submitted by physicians, healthcare consumers, lawyers amongst others.

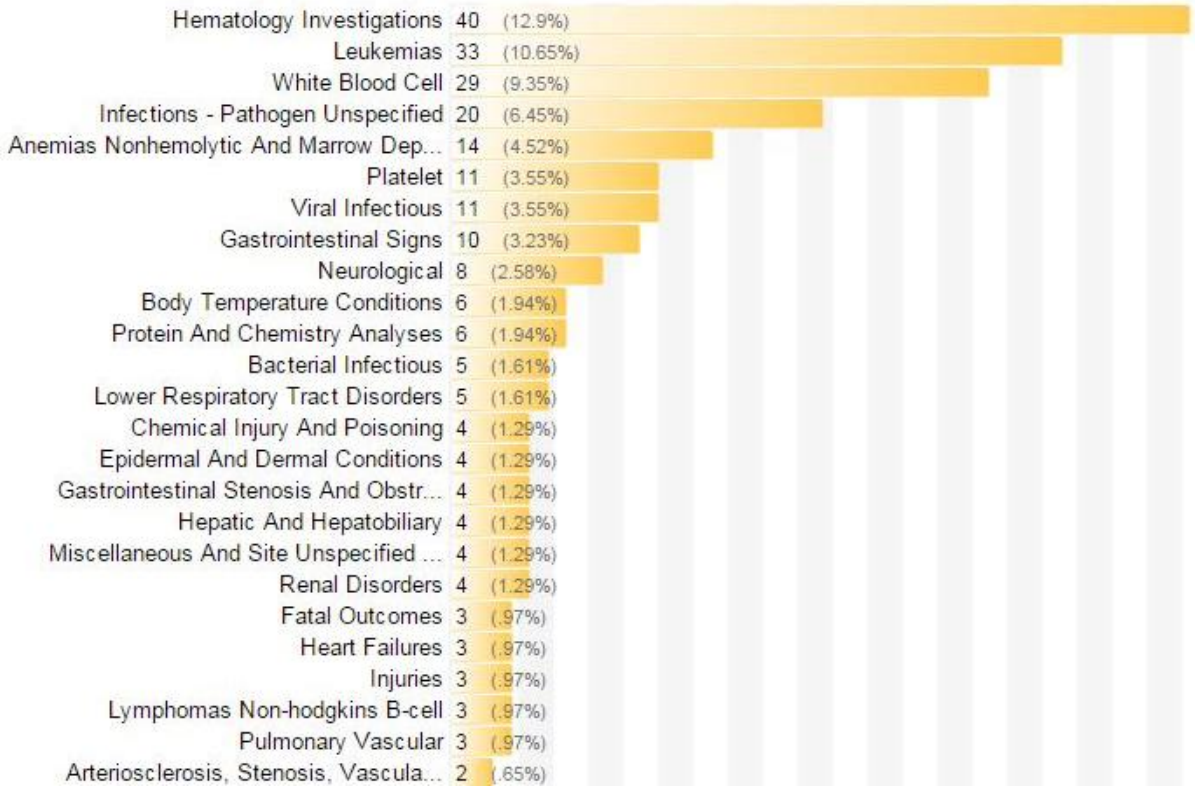

Chart 26 – Individual cases sorted by reactions groups, submitted for Ibritumomab Tiuxetan and other drugs with same active substance (Q1 2004 – Q3 2012) *in* DrugCite.com (www.drugcite.com)

(data extracted on 25-Nov-2014)

The chart above displays, for each reaction group, the number of cases reported where the drug was flagged as a suspect drug causing the adverse event. All percentages (%) are the cases reported for a specific reaction group within the sum up of all reported cases in all reactions groups (percent does not represent rate related to drug utilization).

U.S. Top 10 of Individual Cases (by reactions groups):

1. Hematology Investigations (e.g. Haemoglobin/White Blood Cell Count) (40 cases)
2. Leukemias (33 cases)
3. White Blood Cell (e.g. Febril Neutropenia; Leukopenia) (29 cases)
4. Infections – Pathogen Unspecified (20 cases)
5. Anemias Nonhemolytic And Marrow Depression (14 cases)
6. Platelet (e.g. Thrombocytopenia) (11 cases)
7. Viral Infections (11 cases)
8. Gastrointestinal Signs (10 cases)
9. Neurological (e.g. Syncope; Dizziness; Somnolence) (8 cases)
10. Body Temperature Conditions (6 cases)

- ***Comparison of Adverse Events reported in EU and in the U.S.***

Following the analysis of the Adverse Events previously presented, it became clear that there were some differences between EU data and U.S. data. In order to have a visually enhanced perception of such differences, it was decided to create a cumulative chart which would aggregate all the data previously presented:

- Post Marketing EU data (Blue bars in the cumulative chart)
- Post Marketing U.S. data (Orange bars in the cumulative chart)
- EU data as per Initial documents for MA (MedDRA SOCs highlighted in yellow in the cumulative chart)

For aggregation purposes, a common classification had to be used and so MedDRA SOC was the selected classification for the cumulative chart below presented.

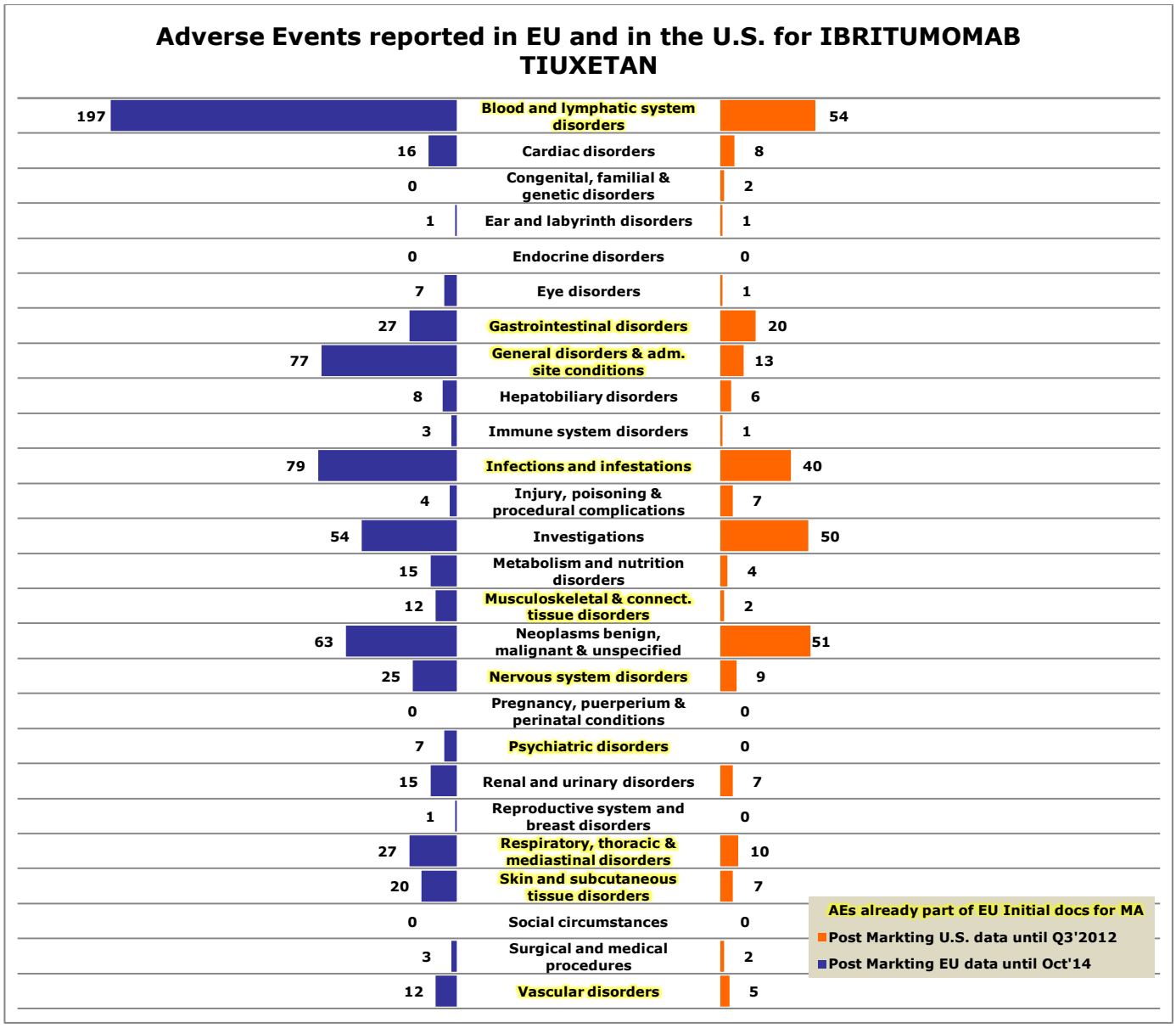

Chart 27 – Individual cases sorted by reactions groups (MedDRA 17.1 SOC), submitted for IBRITUMOMAB TIUXETAN prior and post MA in Europe and post MA the United States

Comments to IBRITUMOMAB TIUXETAN cumulative chart:

1. SOCs included in EU pre-MA reports vs SOCs included in EU post-MA reports

Main discrepancies: There are cases of SOCs with a considerable number of AE reports (>15) in post-MA and which were not foreseen by pre-MA clinical trials data. These include*: Cardiac disorders*; *Investigations* & *Neoplasms benign, malignant and unspecified (including cysts and polyps)*.

2. SOCs included in EU post-MA reports vs. SOCs included in U.S. post-MA reports

Although there are some discrepancies in the main SOCs referred on EU post-MA reports and U.S. post-MA reports and these are not very relevant in the global outcome of the chart.

Small discrepancies: Having EU data as reference, there were some U.S. bars which were expected to be bigger but are not: these include *General disorders and administration site conditions.*

In general, it was observed that Ibritumomab Tiuxetan had a similar profile of AEs reported post-MA in EU and in the U.S.

2. Comparison of AEs reported for anti-CD20 mAbs

In the previous analysis of the AEs reported for each anti-CD20 mAb it was visible that there were some discrepancies in the AEs reported pre and post MA as well as some discrepancies in AEs reported in Europe and the United States.

Following those mAb-specific analysis, it becomes important to see how the AEs are being reported throught anti-CD20 mAbs class.

The table below intends to provide a global picture on the ranking of mostly reported AEs for anti-CD20 mAbs. For comparison purposes, there was the need to chose a common data set and, for that reason, the table only comprises the EU reports post-MA.

Table 29 – Comparison of most commonly reported AEs for anti-CD20 mAbs (post-marketing EU data)

MedDRA 17.1 System Organ Class (SOC)	Post-Marketing EU data – ranking of most reported AEs			Comments
	RITUXIMAB	OFATUMUMAB	IBRITUMOMAB TIUXETAN	
Blood and lymphatic system disorders	3rd	3rd	1st	Most reported AEs for all anti-CD20 mAbs.
Cardiac disorders	10th	--	10th	
Gastrointestinal disorders	8th	8th	7th	Least reported AEs for all anti-CD20 mAbs.
General disorders and administration site conditions	2nd	1st	3rd	Most reported AEs for all anti-CD20 mAbs.
Immune system disorders	9th	--	--	
Infections and Infestations	1st	2nd	2nd	Most reported AEs for all anti-CD20 mAbs.
Injury, poisoning and procedural complications	--	10th	--	
Investigations	7th	5th	5th	Transversally reported AEs for all anti-CD20 mAbs.
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	--	--	4th	
Nervous system disorders	5th	7th	8th	Transversally reported AEs for all anti-CD20 mAbs.
Respiratory, thoracic and mediastinal disorders	4th	4th	6th	Transversally reported AEs for all anti-CD20 mAbs.
Skin and subcutaneous tissue disorders	6th	6th	9th	Transversally reported AEs for all anti-CD20 mAbs.
Vascular disorders	--	9th	--	

NOTE: The colours represent the ranking of reported AEs. **GREEN** represents the mostly reported AEs whereas **RED** the least reported AEs.

According to the above table, anti-CD20 mAbs seem to have a quite similar profile of AEs reported in EU following MA. There are 5 out of 13 (≈38%) SOCs above listed which are not transversally applicable to all this class mAbs. This means that at least 62% of profile is similar in terms of AEs mostly reported.

There were some differences in anti-CD20 mAbs, including the type of mAb (chimeric and human) and the configuration of mAbs (1 mAb is linked the monoclonal antibody to the amino directed bifunctional chelate MX-DTPA), but those differences did not had a major impact on the general outcome of AEs mostly reported to each mAb.

The similar profile seen throught the anti-CD20 mAbs class may be due to a number of factors, including similar treatment indications and equal administration route: intravenous infusion. The similarities within anti-CD20 mAbs class seem to be aligned with the concept of AEs being correlated to mAbs mechanism of action.

For the particular case of anti-CD20 mAbs, the mostly reported AEs were i) *Infections and Infestations*, ii) *General disorders and administration site conditions* as well as iii) *Blood and lymphatic system disorders* and all these are according to the expectation.

While *General disorders and administration site conditions* are antibody-related AEs which generally occur for every mAb; *Infections and Infestations* & *Blood and lymphatic system disorders* can be correlated to the specific Mechanism of Action of anti-CD20.

Similar to anti-TNFα, anti-CD20 have immunosuppressive-like reactions because they reduce B cells and affect the immunity. Their impact on the immune system can result in greater chances for *Infections and Infestations* to arise.

On what regards *Blood and lymphatic system disorders*, these disorders are closely related to the mechanism of action of this restricted class of mAbs.

CD20 is found on both normal and malignant B cells [116]. B cells, in turn, circulate continuously between the blood and lymph [36]. It is predictable that the reduction of B cells, mediated by anti-CD20 mAbs, can contribute to a greater number of *Blood and lymphatic system disorders*.

I. Comparison of AEs reported through all mAbs classes– anti-TNFα, VEGF and CD20

Following the previous analysis which comprised the investigation of AEs reported for a specific mAb and the comparison of AEs reported through each mAb class, it becomes clear that although some discrepancies are found, most mAbs classes have at least half similar AEs profile within the class.

Based on those findings, it was interesting to see if the AEs profiles which were observed to be similar within the class, were also similar at a higher level: through different mAbs class. The table below intends to provide a macro global picture on the ranking of mostly reported AEs for all mAbs class– anti-TNFα, VEGF and CD20.

Once again, for comparison purposes, a common data set had to be chosen and, for that reason, the table only comprises the EU reports post-MA.

Table 30 – Comparison of most commonly reported AEs in the 3 mAbs classes (post-marketing EU data)

MedDRA 17.1 System Organ Class (SOC)	Post-Marketing EU data – rating of AEs mostly reported								
	anti-TNFα mAbs				anti-CD20 mAbs			anti-VEGF mAbs	
	ADALIMUMAB	INFLIXIMAB	GOLIMUMAB	CERTOLIZUMAB	RITUXIMAB	OFATUMUMAB	IBRITUMOMAB TIUXETAN	BEVACIZUMAB	RANIBIZUMAB
Blood and lymphatic system disorders	--	--	--	--	3rd	3rd	1st	7th	--
Cardiac disorders	--	--	--	--	10th	--	10th	--	5th
Gastrointestinal disorders	3rd	3rd	6th	2nd	8th	8th	7th	2nd	--
General disorders and administration site conditions	2nd	2nd	2nd	3rd	2nd	1st	3rd	1st	2nd
Eye disorders	--	--	--	--	--	--	--	10th	1st
Immune system disorders	--	--	--	--	9th	--	--	--	--
Infections and Infestations	1st	1st	1st	1st	1st	2nd	2nd	6th	3rd
Injury, poisoning and procedural complications	10th	4th	8th	9th	--	10th	--	--	7th
Investigations	9th	10th	10th	10th	7th	5th	5th	8th	6th
Musculoskeletal and connective tissue disorders	5th	8th	5th	5th	--	--	--	--	--
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	4th	9th	9th	--	--	--	4th	9th	10th
Nervous system disorders	6th	7th	3rd	6th	5th	7th	8th	4th	4th
Renal and urinary disorders	--	--	--	--	--	--	--	--	--
Reproductive system and breast disorders	--	--	--	--	--	--	--	3rd	--
Respiratory, thoracic and mediastinal disorders	8th	5th	7th	8th	4th	4th	6th	--	9th
Skin and subcutaneous tissue disorders	7th	6th	4th	4th	6th	6th	9th	--	--
Surgical and medical procedures	--	--	--	7th	--	--	--	--	--
Vascular disorders	--	--	--	--	--	9th	--	5th	8th

NOTE: The colours represent the ranking of reported AEs. **GREEN** represents the mostly reported AEs whereas **RED** the least reported AEs.

General comments on the global AEs comparison: Some interesting findings can be observed in the global table comprising all mAbs classes – anti-TNFα, anti-CD20 and anti-VEGF.

At a macro level, the profiles have similarities and some differences and it is interesting to see that the AEs here reported can be divided in three main types:

1. antibody-related AEs: *General disorders and administration site conditions* are transversally reported throughout all mAbs classes. These occur for every mAb, regardless of the target involved.
2. class-related AEs: *Infections and Infestations* are observed for all immunosuppressor agents: anti-TNFα and anti-CD20 mAbs.
3. restricted class-related AEs: *Blood and lymphatic system disorders* observed for anti-CD20 mAbs; *Vascular disorders* for anti-VEGF and *Gastrointestinal disorders* for anti-TNF-α mAbs.

As per above table, there are not many reports of AEs classified as *Neoplasms benign, malignant and unspecified (incl cysts and polyps)*. It is yet to discover if these low numbers will be maintained or if they will change alongside with the long-term exposure to these mAbs.

FINAL REMARKS

Although mAbs were, at first, thought to be the closest thing to "ideal drugs" due to their specificity and efficacy, latter in usage it became clear that mAbs can have adverse events too.

Furthermore, it was understood that many of those adverse events could not be expected by the results obtained in non-clinical studies. If from one point of view, mAbs have in their favour higher specificity, which gives them great efficacy results and prevents adverse events due to action in non-intended targets; this same characteristic can also have some cons [4, 126]. It is well known that many mAbs operate through the immunosuppressive effect [127], which is useful for the treatment of various conditions, such as the prevention of graft rejection, cancer and auto-immune diseases including rheumatoid arthritis, Crohn disease or multiple sclerosis. The problem is that mAbs' targets have many times critical roles for the well function of the body and their suppression can compromise vital functions. A classic reported example are the anti-TNFα mAbs that by suppressing the target to reduce clinical signs and symptoms of chronic inflammatory diseases, also revealed to compromise host infections' defence [126]. These occurrences brought new concerns and, more than proving that mechanism of action plays an important role for adverse events occurrences, it is important to understand how this happens and what can be predicted upfront.

The goal of this project was to correlate mAbs' adverse events with their specific mechanism of action. For that purpose, and considering the extensive number of mAbs available in Europe, it was decided to study only 3 classes of mAbs well-established in the market: anti-TNFα, anti-VEGF and anti-CD20. The adverse events collected and analysed included European and United States data as well as data available pre and post marketing authorisations.

Although with some exceptions, it was observed that the adverse events anticipated prior marketing authorisation were similar to the ones observed in post marketing and it was also observed that the European data was similar to the United States data.

On what regards adverse events correlation with the mechanism of action, it was confirmed that one influences the other. There were reports of **antibody-related adverse events** occurring for all mAbs (e.g. *General disorders and administration site conditions*); **class-related adverse events** occurring for specific classes of mAbs (e.g. *Infections and Infestations*) as well as **restricted class-related adverse events** (e.g. *Blood and lymphatic system disorders* observed for anti-CD20 mAbs & *Vascular disorders* for anti-VEGF mAbs).

For restricted class-related adverse events, it was also observed that there are additional key factors that cannot be excluded when analysing the safety profiles of mAbs – administration routes, mAbs structural configurations and profile of patients receiving those mAbs can also impact significantly the adverse events reported in clinic. This was the case for anti-VEGF mAbs.

All the class-related and restricted-class related adverse events confirmed that mAbs' mechanism of action plays a crucial role in the adverse events later reported in clinic. The characterisation of each mAb specificities together with a precise understanding of the mAbs mechanism of action can be the basis for mAbs safety profile characterisation.

APPENDIX 1 – mAbs UNDERLYING DISEASES

I. Underlying diseases of anti-TNFα mAbs

Table 31 – Underlying diseases of mAbs targeted to TNFα

anti-TNFα mAbs with that indication	Indications	Diseases Characterization	References
☒ **Adalimumab** ☒ **Infliximab** ☒ **Golimumab** ☒ **Certolizumab peg.**	***Rheumatoid Arthritis (RA)***	– chronic multisystemic inflammatory disease with prominent autoimmune features – characterised by chronic inflammation of several joints and is often complicated by the involvement of internal organs – principal characteristic is persistent inflammatory synovitis, but it displays also a variety of clinical manifestations as defined by the American College of Rheumatology: • Morning stiffness (≥ 1 hr) • Swelling of joints (≥ 3) • Swelling of soft tissue of hand joints (PIP*, MCP*, wrist) • Symmetrical soft tissue swelling • Subcutaneous nodules • Serum rheumatoid factor • Radiographic evidence of erosion or periarticular osteopenia in hand or wrist joints *PIP = proximal interphalangeal; MCP = metacarpophalangeal Criteria 1 to 4 must be continuous for 6 weeks. A diagnosis of rheumatoid arthritis requires that 4 of the 7 criteria be fulfilled.	[69, 77]
☒ **Adalimumab** ☐ Infliximab ☐ Golimumab ☐ Certolizumab peg.	***Polyarticular Juvenile Idiopathic Arthritis (Poly-JIA)***	– autoimmune disease with a complex genetic predisposition – onset occurring in children under the age of 16 years – most common rheumatic disease of childhood and an important cause of disability in children – The onset of JIA is characterized by three primary modes: 1. pauciarticular (<5 joints) – the most frequent mode, observed in 50% of patients; 2. polyarticular (≥5 joints) – observed in 30% of patients; 3. systemic arthritis (≥1 joint with fever and rash) – observed in 10% to 20% of patients	[128]

anti-TNFα mAbs with that indication	Indications	Diseases Characterization	References
☒ **Adalimumab** ☐ Infliximab ☐ Golimumab ☒ **Certolizumab peg.**	***Axial spondyloarthritis (AxSpA)***	– refers to spondyloarthropathy with predominantly axial involvement and comprises: • sub-group of ankylosing Spondylitis • sub-group with little or no changes on plain radiographs, referred to as non-radiographic Axial Spondylitis (nr-AxSpA) – Assessments in Spondyloarthritis International Society (ASAS) criteria for classification of AxSpA, are: • Back pain of ≥3 month duration at age of onset <45 • and either of the three is true for the subject: 1. active (acute) inflammation on MRI highly suggestive of sacroiliitis associated with SpA and at least 1 clinical features* 2. definitive sacroiliitis (grade ≥2 bilaterally or ≥3 unilaterally) on x-ray and at least 1 clinical features* 3. HLA B27 positive and has at least 2 clinical features* * Clinical features e.gs.: *inflammatory back pain; arthritis; enthesitis; uveitis; dactylitis; psoriasis; elevated CRP; family history for SpA*	[129, 130]
☒ **Adalimumab** ☒ **Infliximab** ☒ **Golimumab** ☒ **Certolizumab peg.**	***Ankylosing Spondylitis (AS)***	– If definite radiographic sacroiliitis on plain X-rays is present, the disease can most likely be classified as Ankylosing Spondylitis (AS) – modified New York criteria for AS (mNY) requires: • x-ray findings of sacroiliitis grade ≥2 bilaterally or grade 3-4 unilaterally • and at least 1 of the following clinical criteria: 1. Low back pain and stiffness for more than 3 months that improves with exercise but is not relieved by rest. 2. Limitation of motion of the lumbar spine in the sagittal and frontal planes. 3. Limitation of chest expansion relative to normal values correlated for age and sex – chronic inflammatory disease of unknown etiology, which involves the spine and tendons – characterised by progressive, stiffening of the sacroiliac, intervertebral and costovertebral joints and leading to bony ankylosis – systemic rheumatic disease and may also effect enthesis (tendon insertions) and peripheral joints, as well as other organs such as the eyes, heart, and lungs – may also have nonskeletal manifestations, including uveitis, carditis, pulmonary fibrosis, and cardiac conduction abnormalities – is associated with the presence of the HLA-B27 allele	[72, 77, 80, 129]

anti-TNFα mAbs with that indication	Indications	Diseases Characterization	References
☒ **Adalimumab** ☒ **Infliximab** ☒ **Golimumab** ☒ **Certolizumab peg.**	***Psoriatic Arthritis (PsA)***	– chronic, inflammatory, usually rheumatoid factor (RF)-negative arthritis associated with psoriasis, which is classified within the group of the spondyloarthritis – the combination of joint and skin manifestations of PsA can have a profound impact on patient function, well-being, and health-related quality of life – usually involves multiple peripheral joints, the axial skeleton, sacroiliac joints, fingernails, and enthuses – presentation of PsA has been categorised into 5 overlapping clinical patterns: 1. oligoarthritis 2. polyarthritis 3. arthritis of distal interphalangeal (DIP) joints 4. Spondylitis 5. arthritis mutilans – More than one-half of the patients with PsA may have evidence of erosions on X-rays, and up to 40% of the patients may develop severe, erosive arthropathy	[77, 131-135]
☒ **Adalimumab** ☒ **Infliximab** ☐ Golimumab ☐ Certolizumab peg.	***Psoriasis (Ps)***	– common, chronic immunologic disease – characterised by marked inflammation and thickening of the epidermis resulting in thick, scaly plaques involving the skin – life-long disease often diagnosed early in life – not a life-threatening disease, but in relation to the extent and location of the plaques can lead to serious social and psychological impairment – Psoriasis may be classified as plaque psoriasis, guttate psoriasis, erythrodermic psoriasis, generalised pustular and localised pustular psoriasis, and inverse or intertriginous psoriasis (plaque psoriasis is the most common form seen in 75 to 80% of psoriasis patients). – Approximately 6% up to 40% of Psoriasis patients may have PsA associated	[136, 137]
☒ **Adalimumab** ☒ **Infliximab** ☐ Golimumab ☒ **Certolizumab peg.**	***Crohn's Disease (CD)***	– chronic inflammatory disease of the bowel, which appears periodically with a varying course – characterized by segmental transmural inflammation and granulomatous changes. – affects primarily the distal small intestine and the colon, but it may affect also any part of the gut	[72, 76, 77]

anti-TNFα mAbs with that indication	Indications	Diseases Characterization	References
☒ **Adalimumab** ☒ **Infliximab** ☒ **Golimumab** ☐ Certolizumab peg.	***Ulcerative Colitis (UC)***	– first of the 2 primary forms of idiopathic inflammatory bowel disease – chronic, relapsing inflammatory disease of the rectum and/or large intestine – characterized by inflammation and ulceration of the mucosal and submucosal intestinal layers – about 40 to 50% of patients have disease limited to the rectum and rectosigmoid, 30 to 40% have disease extending beyond the sigmoid but not involving the whole colon, and 20% have a total colitis – diagnosis is established after colonoscopy and histology of colonic mucosa – can be considered an autoimmune disease, harbouring in genetically susceptible individuals	[72, 138-140]

II. Underlying diseases of anti-VEGF mAbs

Table 32 – Underlying diseases of mAbs targeted to VEGF

anti-VEGF mAbs with that indication	Indications	Diseases Characterization	References
☒ **Bevacizumab** ☐ Ranibizumab	***Metastatic Carcinoma of the Colon or Rectum (mCRC)***	– major public health problem – second most common malignancies in both men and women – Approximately 30% of all patients with CRC have metastatic disease at diagnosis, and 50% of early-stage patients will eventually develop metastatic or advanced disease	[99]
☒ **Bevacizumab** ☐ Ranibizumab	***Metastatic breast cancer (mBC)***	– Most common cancer in women worldwide. The median survival for patients with metastases at diagnosis is around 2-3 years with <20% still alive at 5 years	[141]
☒ **Bevacizumab** ☐ Ranibizumab	***Non-Small Cell Lung Cancer (NSCLC)***	– NSCLC represents about 80% of lung cancer – The most common histologies are epidermoid or squamous cell carcinoma (30-35%), adenocarcinoma (40-45%), and large cell carcinoma (<10%). • These histologies are often classified together because approaches to diagnosis, staging, prognosis, and treatment are similar. – many patients are diagnosed an advanced stage of the disease (approximately 30% locally advanced and 40% metastatic disease) with the remainder (25-30%) presenting with early stage – the 5-year survival rate is only about 15%	[142]
☒ **Bevacizumab** ☐ Ranibizumab	***Metastatic Renal Cell Carcinoma (mRCC)***	– adenocarcinoma originating in the renal cortex – accounts for about 2% of all solid tumours, and 90-95% of tumours arising from the kidney – the four main histological subtypes of RCC are, according to their degree of increasing aggressiveness: 1. oncocytic 2. chromophobic 3. chromophilic 4. clear-cell – Commonly, RCC presents as a mixture of these different histological subtypes. – the 5-year survival rate is only about 5 -15%	[143]

anti-VEGF mAbs with that indication	Indications	Diseases Characterization	References
☒ **Bevacizumab** ☐ Ranibizumab	***Epithelial ovarian, fallopian tube and primary peritoneal cancer***	– one of the most common gynaecological – affects globally nearly a quarter of a million women each year and it is the 8th leading cause of cancer death in women and the 7th leading cause of cancer death among women – The most common group of ovarian cancers that arise in the epithelium are: • epithelial ovarian cancer (EOC) • fallopian tube cancer (FTC) • primary peritoneal cancer (PPC). – Because the disease tends to be asymptomatic in early stages, or associated with vague, non-specific symptoms, the majority of patients are diagnosed with advanced stage disease	[144, 145]
☒ **Bevacizumab** ☐ Ranibizumab	***Glioblastoma***	– rapidly progressing cancer that invades brain tissue and can impact physical activities and mental abilities	[146]
☒ **Bevacizumab** ☐ Ranibizumab	***Cervical Cancer***	– grows in the tissues of the lower part of the uterus known as the cervix – commonly occurs when human papillomaviruses (HPV), a virus that spreads through sexual contact, cause cells to become cancerous	[147]
☐ Bevacizumab ☒ **Ranibizumab**	***Age-related macular degeneration (AMD)***	– progressive degenerative macular disease attacking the region of highest visual acuity (VA), the macula – major cause of vision loss in the elderly population – although the disease rarely results in complete blindness and peripheral vision may remain unaffected, central vision is gradually blurred, severely affecting ordinary daily activities – AMD is classified into two different types: • non-exudative (or dry) form (most prevalent ≈ 90% cases) • exudative (wet or neovascular) form – angiographic classification of AMD lesions includes the determination of • lesion size • proportion of the entire AMD lesion that consists of 'classic' and 'occult' choroidal neovascularisation (CNV)	[107]

anti-VEGF mAbs with that indication	Indications	Diseases Characterization	References
☐ Bevacizumab ☒ **Ranibizumab**	***Diabetic macular oedema (DME)***	– characterised by swelling of the central part of the retina, the macula, which mediates high-resolution visual acuity (VA) – one of the most sight-threatening complications in diabetes mellitus • estimated to occur in around 10% of the diabetic population, with a high prevalence of 30% in patients with more than 25-year history of diabetes. – DME arises from breakdown of the blood retinal barrier (BRB), leading to leakage of fluid and plasma constituents in the surrounding retina, resulting in retinal oedema – The natural progression of DME leads to vision loss of two or more lines (≥ 10 letters) of VA within 2 years in approximately 50% of patients	[148]
☐ Bevacizumab ☒ **Ranibizumab**	***Macular oedema secondary to Retinal Vein Occlusion (RVO)***	– second leading cause of blindness for patients with retinal vascular disease, following diabetic retinopathy – There are two main types of RVO, depending on the site of the vein occlusion: • branch retinal vein occlusion (BRVO) • central retinal vein occlusion (CRVO) – In addition, when the occlusion is located in one of the 2 branches after the division of the central vein the occlusion is classified as a hemiretinal vein occlusion (HRVO). – most common presenting symptom of RVO is an abrupt, painless decrease of central visual acuity (VA) which varies in severity in BRVO and CRVO. – Untreated eyes with CRVO generally have poor VA (less than 20/40) which declines further over time	[149]

anti-VEGF mAbs with that indication	Indications	Diseases Characterization	References
☐ Bevacizumab ☒ **Ranibizumab**	***Choroidal Neovascularisation (CNV) Secondary to Pathologic Myopia***	– CNV secondary to Pathologic Myopia is considered one of the major causes of legal blindness in several countries and the leading cause of visual impairment in young patients worldwide. – In Pathologic Myopia the axial length of the eyeball is abnormally elongated (> 26 mm), which is associated with high myopia refractive errors and changes of the posterior pole of the eye such as posterior staphyloma, atrophy of the retinal pigment epithelium, Bruch's membrane cracks, subretinal haemorrhage, retinal detachment, and CNV. – CNV is considered the most vision threatening complication in patients with Pathologic Myopia. – During the natural course of CNV secondary to Pathologic Myopia, patients progressively lose VA at a rate of approximately 10 to 15 letters (2 to 3 lines) over 2 years. – The prevalence of CNV in patients with Pathologic Myopia is high in patients under the age of 50 years; which contributes for a profound impact on the productivity of this working age group.	[150]

III. Underlying diseases of anti-CD20 mAbs

Table 33 – Underlying diseases of mAbs targeted to CD20

anti-CD20 mAbs with that indication	Indications	Diseases Characterization	References
☒ Rituximab ☐ Ofatumumab ☒ Ibritumomab Tiuxetan	***Follicular Lymphoma NHL(Non-Hodgkin's Lymphoma*)***	– Follicular lymphomas account for 20-25% of all lymphomas and are the second most common subtype of NHL – mostly occur on middle-aged and older people, with a median age at diagnosis of approximately 60 years. – Most of the time, this lymphoma occurs in many lymph node sites in the body, as well as in the bone marrow – often slow-growing and respond well to treatment, but they are hard to cure – these lymphomas may not require treatment when they are first diagnosed and treatment may be delayed until the lymphoma is causing problems – Over time, about 1 in 3 follicular lymphomas turns into a fast-growing diffuse B-cell lymphoma.	[116, 151-153]

anti-CD20 mAbs with that indication	Indications	Diseases Characterization	References
☒ **Rituximab** ☐ Ofatumumab ☐ Ibritumomab Tiuxetan	***Diffuse Large B cell NHL***	– mostly occurs in older people (the average age is mid-60s) – usually starts as a quickly growing mass in a lymph node deep inside the body, such as in the chest or abdomen, or in a lymph node, such as in the neck or armpit • It can also start in other areas such as the intestines, bone, or even the brain or spinal cord – Some of these lymphomas are localized, which makes it easier to treat	[153]
☒ **Rituximab** ☒ **Ofatumumab** ☐ Ibritumomab Tiuxetan	***Chronic lymphocytic leukaemia (CLL)***	– most common form of adult leukaemia comprising 30% of all adult leukaemias – The median age at first diagnosis is in the range of 65-70 years and men are affected more often than women. Over the years, the incidence has increased in younger patients and now about one-third of CLL patients are younger than 55 years at diagnosis. – characterized by an extremely variable clinical course and prognosis depending on disease stage and presence. The two clinical staging systems developed by Rai and Binet are widely used and are an accepted prognostic tool	[154]
☒ **Rituximab** ☐ Ofatumumab ☐ Ibritumomab Tiuxetan	***Rheumatoid arthritis (RA)***	– B cells both responds to, and produce, chemokines and cytokines that promote leukocyte infiltration into the joints, formation of ectopic lymphoid structures, angiogenesis, and synovial hyperplasia that characterize the pathophysiological changes observed in the rheumatoid joint – In some patients, rheumatoid synovitis is associated with the formation of complex lymphoid microstructures, to the extent that the rheumatoid process induces the formation of T cell-B cell follicles with germinal centre reactions in the synovium of affected joints. Thus, B-cell targeted therapy can play a variety of roles in RA through reduction in B-cell effector cells, as well as downstream effects on other cells that participate in the inflammatory response	[155]

anti-CD20 mAbs with that indication	Indications	Diseases Characterization	References
☒ **Rituximab** ☐ Ofatumumab ☐ Ibritumomab Tiuxetan	***Granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA)***	– GPA and MPA are both associated with anti-neutrophil cytoplasmic antibodies (ANCAs) and are therefore referred to collectively as ANCA-associated vasculitis (AAV). – Two types of ANCA have been identified in GPA and MPA, defined by the antigenicity against two different self-antigens which are the endogenous enzymes; MPO (myeloperoxidase) and PR3 (proteinase-3) that are present in neutrophilic lymphocytes. – ANCA-serology has a different distribution between GPA and MPA. GPA is mainly associated with PR3-ANCA (95% of the cases), whereas MPA is more frequently associated with MPO-ANCA (75% of MPA patients) – GPA prominent features: crusting granulomata in the ear, nose and throat area and alveolar bleeding. Segmental, necrotizing glomerulonephritis may occur in about 50% of the cases. – MPA prominent features: glomerulonephritis – In both GPA and MPA, vasculitis may be extended to other organs like the skin, nervous system (specifically vaso nervorum of peripheral nerves), heart and gastrointestinal tract (mesenterium). – CNS involvement is relatively rare for both disorders. – Systemic features (fever, arthritis) are more common in MPA	[156]

*** <u>Non-Hodgkin's Lymphoma (NHL):</u>**

Non-Hodgkin's Lymphoma (NHL) is t he most common of the malignant lymphoid neoplasms.

NHL is the common name for a cluster of related but individual diseases, which have neoplastic transformation of a lymphoid cell as the common denominator. 85% of the NHLs are derived from a B-lymphocyte and 15% from a T-lymphocyte. **Follicular Lymphoma** and **Diffuse Large B cell Non-Hodgkin's Lymphoma** are included in B- cell lymphomas.

There have been some improvements and changes in the system broadly used for NHL's classification. Currently the main system used is the WHO classification. Translation of diagnoses within the systems is possible for most but not all classes. The WHO system groups lymphomas based on how they look under a microscope, the chromosome features of the lymphoma cells, and the presence of certain proteins on the surface of the cells.

The WHO classification recognizes 13 individual B-cell lymphomas (with several further subdivisions), which form a clinical spectrum from the low-grade lymphocytic and follicular lymphomas with a medium survival of 10-15 years at one end, and the high grade lymphoblastic lymphomas with a medium survival of months at the other end of the spectrum [116, 153].

The course of disease often contains several relapses that are manageable but ultimately most patients will develop chemoresistant lymphoma often with a transformation to a large cell more malignant NHL [116].

The disease generally manifests with hypertrophy of peripheral and deep lymph nodes and splenomegaly, together with bone marrow infiltration. Histological transformation to more aggressive non-Hodgkin's lymphoma (NHL) with poor prognosis has been documented to occur in 20-80% of patients over time. Also, the likelihood of resistant lymphoma or occurrence of secondary malignancies such as myelodysplastic syndrome/acute myeloid leukaemia (MDS/AML) increases with time and with the number, choice and intensity of previous treatments [151, 152].

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